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CONTENTS

STUDIES ON THE PAIN-SENSIBILITY OF ARTERIES. I. SOME OBSERVATIONS ON THE PAIN-SENSIBILITY OF ARTERIES. *Robert M. Moore and Ruth E. Moore* 259

STUDIES ON THE PAIN-SENSIBILITY OF ARTERIES. II. PERIPHERAL PATHS OF AFFERENT NEURONES FROM THE ARTERIES OF THE EXTREMITIES AND OF THE ABDOMINAL VISCERA. *Robert M. Moore and Albert O. Singleton, Jr.* 267

A QUANTITATIVE STUDY OF THE CHANGES IN POWER DURING MUSCULAR CONTRACTION. *H. C. Stevens and J. M. Snodgrass* 276

STUDIES ON NERVE METABOLISM. VII. THE RÔLE OF LACTIC ACID. *T. H. Chang and R. W. Gerard* 291

EFFECTS OF ADRENAL MEDULLA AND THYROID ON THE RESPIRATORY METABOLISM OF PANCREATIC DIABETES. *G. C. Ring and C. W. Hampel* 298

THE OXYGEN CONSUMPTION OF STIMULATED NERVE. *Francis O. Schmitt* 303

THE RELATION BETWEEN THE AFTER-POTENTIAL AND OXIDATIVE PROCESSES IN MEDULLATED NERVE. *Francis O. Schmitt and Herbert S. Gasser* 320

UTERINE MOTILITY IN HYPOPHYSECTOMIZED AND IN PREGNANT RABBITS. *Samuel R. N. Reynolds and Warfield M. Firor* 331

STUDIES ON THE PHYSIOLOGY OF SLEEP. VII. THE EFFECT OF THE POSITION OF THE BODY AND OF SLEEP ON RECTAL TEMPERATURE IN MAN. *N. Kleitman and A. Doktorsky* 340

THE EFFECT OF INTRAVENOUS ADMINISTRATION OF LECITHIN AND SODIUM CHLORIDE ON BLOOD SUGAR VALUES. *D. E. Gregg* 344

THE EFFECT OF SHORT RADIO WAVES AND HEAT ON THE ELASTICITY OF THE AORTA. *R. W. Heinle and K. R. Phelps* 347

THE EFFECT OF SHORT RADIO WAVES ON PERFUSED CATS' HEARTS. *R. W. Heinle and K. R. Phelps* 349

BONE MARROW VOLUME IN ADULT DOGS. *Edna Fairman and George H. Whipple* 352

EFFECT OF RESPIRATION ON CARDIAC OUTPUT. *J. A. E. Eyster and Earl V. Hicks* 358

OBSERVATIONS ON THE BLOOD OF NORMAL HUMAN SUBJECTS DURING FETAL LIVER FEEDING. *Orville S. Walters and Parke H. Woodard* 364

THE GASTRIC HUNGER MECHANISM. IV. THE INFLUENCE OF EXPERIMENTAL ALTERATIONS IN BLOOD SUGAR CONCENTRATION ON THE GASTRIC HUNGER CONTRACTIONS. *Michael G. Mulinos* 371

CHANGES IN THE SKELETAL MUSCLE OF THE RAT FOLLOWING DENERVATION. *H. M. Hines and G. C. Knowlton* 379

THE RELATION BETWEEN BLOOD OSMOTIC PRESSURE AND INSENSIBLE WEIGHT LOSS. *Alfred Gilman and Henry G. Barbour* 392

EFFECTS OF ADRENALECTOMY AND CORTICO-ADRENAL EXTRACT ON RENAL EXCRETION AND TISSUE FLUIDS. *H. Silvette and S. W. Britton* 399

A COMPARISON OF THE CLEARANCES OF VARIOUS URINARY CONSTITUENTS. *H. L. White and Betty Monaghan* 412

THE CIRCULATORY EFFECTS OF ACUTE EXPERIMENTAL HYPERVOLEMIA. *D. E. Gregg and C. J. Wiggers* 423

ACTION OF ACETYCHOLINE ON THE INTESTINE. *Frederick Bernheim* 433

OXIDATION OF ACETYCHOLINE BY TISSUES. *Frederick Bernheim and Mary L. C. Bernheim.* 438

SIMULTANEOUS STUDY OF THE CONSTITUENTS OF THE SWEAT, URINE AND BLOOD; ALSO GASTRIC ACIDITY AND OTHER MANIFESTATIONS RESULTING FROM SWEATING. X. BASIC METALS. *G. A. Talbert, C. Haugen, R. Carpenter, and J. E. Bryant* 441

THE EFFECT OF OCCLUSION OF THE CAROTID ARTERIES ON HEART RATE AND RESPIRATORY RATE BEFORE AND AFTER DENERVATION OF THE CAROTID SINUS IN NORMAL DOGS. *C. L. Gemmill, E. W. Overstreet and L. M. Hellman* 443

(Continued on cover 4)

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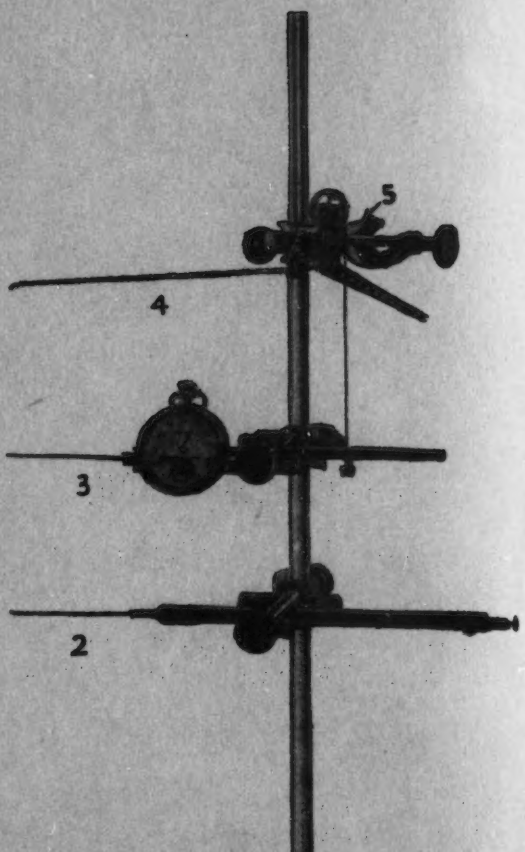

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STUDIES ON THE PAIN-SENSIBILITY OF ARTERIES

I. Some Observations on the Pain-Sensibility of Arteries

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Various observers, including Odermatt (1922), Friedrich (1924), Schilf and Stahl (1925), Dennig (1924, 1925), Abrashanow (1927), and Burget and Livingston (1931), have described the painful effect of the injection of irritants into arteries. No very determined attempt seems to have been made to ascertain the mechanism whereby such pain is produced. In view of the meager knowledge of the sensibility of arteries it seems desirable to report the results of a series of experiments upon intravascular injection of irritants and to attempt to correlate this evidence with observations on other conceivable types of arterial pain-sensibility.

In this laboratory, during the course of experiments in radiographic visualization of arteries, it was noted that in animals anesthetized with sodium amytal to a sufficient degree to permit the performance of laparotomy without notable reflex response, the injection into the femoral artery of a small quantity of a concentrated solution of sodium iodide invariably provoked a marked reflex response. The entire body stiffened and the extremities strained at the leashes; a hyperpnea developed and the pupils became widely dilated; the head was tossed from side to side and there was an accompanying vocalization.

Such activity is an integral part of the external manifestation of the perception of acute pain during conscious states. Woodworth and Sherrington (1904) called attention to its occurrence in response to afferent stimulation after section of the brain-stem below the diencephalon. When appearing in the absence of consciousness it has been termed pseudaffective or quasi-emotional behavior. In the experiments described it was not accompanied by subjective perception of pain since the higher centers had been rendered inactive by the general anesthetic agent. However, it was initi-

ated, we may presume, by afferent impulses which would have evoked pain had the functions of the brain been intact.

This pseudaffective reaction of the amytalized cat has been used in studies to determine the sensibility of arteries to various types of stimulation. Since the reaction does not lend itself readily to mechanical recording methods the results to be reported are based upon simultaneous judgments of two observers as to the absence, presence and intensity of reflex motor response. In each case the response, if present, has been graded according to the following plan: ++++—marked struggling and hyperpnea with vocalization, +++—marked struggling and hyperpnea without vocalization, ++—moderate struggling and hyperpnea, +—definite but slight muscular response and change in respiration, ?—questionable response, and 0—no response.

The majority of the experiments were performed in the cat with occasional observations on the dog. An aqueous solution of sodium amytal was administered by intraperitoneal injection in doses of 50 to 70 mgm. per kgm. for the cat and of 45 to 60 mgm. per kgm. for the dog. A period of 20 to 40 minutes was necessary for induction of anesthesia of moderate depth, providing indifference to ordinary surgical procedure combined with an active corneal reflex. Occasionally a greater amount of the drug was required.

In the majority of experiments the irritant consisted of a concentrated solution of sodium iodide (100 grams of the salt added to 100 cc. of water). Occasionally other irritants were used, including less concentrated solutions of sodium iodide or of potassium iodide, 10 to 30 per cent sodium chloride, 5 to 50 per cent lactic acid, and 10 per cent barium chloride; 0.25 to 0.5 cc. of the irritant was injected. To prevent excessive dilution the artery was occluded temporarily just above the site of injection by tension on an encircling silk thread.

In all over 200 intra-arterial injections of an irritant were made in approximately 100 animals. As a usual result there was a surprisingly strong reflex motor reaction following injection of any of the irritants used into any artery. The sensibility in question is therefore widespread. It is more acute in the arteries to the extremities than in those to visceral organs. Including injections into the abdominal aorta, which were made immediately above its bifurcation, in 93 instances an irritant was injected into an artery supplying the tissues of an extremity. In no case did a pseudaffective reaction fail to appear, whereas in only 16 instances was it of less than three-plus or four-plus intensity. In contrast, in 68 cases an irritant was injected into an abdominal artery of visceral distribution (hepatic, splenic, superior mesenteric, renal); in 20 instances a pseudaffective reaction was absent or but questionably present whereas in only 13 or the remaining 48 cases did it warrant a three-plus or four-plus designation. This greater

sensitiveness of the arteries of the extremities may be due to a more liberal distribution of the afferent nerve-endings concerned.

Among visceral arteries those supplying upper abdominal organs (hepatic, splenic) show, in turn, a more acute sensibility than do the superior mesenteric and renal arteries which supply lower abdominal structures. On the other hand the inferior mesenteric artery provides more intense reactions than do the renal and superior mesenteric vessels. The difference, in this instance, probably results from the liberal anatomoses with the vessels of the lower extremity, by way of which an irritant injected into the inferior mesenteric artery rapidly finds its way into the vascular system of the thigh.

The reaction to the intra-arterial injection of the irritant appeared promptly. Usually muscular movements were becoming evident within 1 to 1.5 second after the beginning of the injection and had become well developed at the end of a two-second period. In the cat vocalization did not begin until the reflex muscular effects were under way, the duration of the entire response varying from 5 seconds in some animals to 30 seconds in others. In dogs the muscular response was slight and the vocalization more prominent.

Throughout the entire study there was evidence of strong stimulation of sensory endings. Even when the reaction was slight the stimulation was of necessity considerable since the animals were anesthetized to a degree which permitted ordinary surgical procedures without notable reflex disturbance. Similarly, in man such strong stimulation results from the injection of sodium iodide into the femoral artery as to necessitate the use of general anesthesia (Brooks, 1924; Singleton, 1928). Such evidence clearly indicates the existence of numerous afferent fibers which subserve pain-sensation, the endings of which are in some manner stimulated when an irritant is injected into an artery.

Whether the irritant acts directly upon sensory nerve-endings is a question which arises. Stretching of smooth muscle and spastic contraction of smooth muscle have been considered important mechanisms in determining visceral pain. It can be said definitely that the stimulation of sensory fibers in the experiments reported was not due to stretching of muscle through forced dilatation of the artery. In repeated trials some non-irritating solution such as normal salt-solution was injected so rapidly as to distend the artery visibly without eliciting any sign of painful stimulation. On the other hand the irritating solutions produced marked reflex reactions when injected drop by drop.

Likewise evidence has been obtained which shows that the afferent stimulation did not take origin from a spasm of the arterial muscle. In animals in which one lower extremity had been sympathectomized by lumbar ganglionectomy the injection of the sodium-iodide solution into the para-

lyzed femoral artery caused, without exception, as immediate and as pronounced a reaction as a similar injection into the artery having normal innervation. In other unilaterally sympathectomized animals the arteries of the lower extremities were visualized by roentgenograms taken immediately after injection of sodium iodide into the abdominal aorta. The paralyzed arteries of the sympathectomized limb were seen to be dilated relative to those of the normal limb at a moment when painful stimulation was in progress (Moore, Williams and Singleton, 1933). Therefore, since neither spasm of smooth muscle nor stretching of smooth muscle is present at the time of sensory stimulation, one is led to conclude that the irritating solution must act directly upon nerve-endings.

A number of experiments have been performed to determine the location of the endings which are affected by such injections. That the receptor endings of cutaneous sensibility are not those affected is shown by the occurrence of quite intense reactions upon injection of arteries of purely visceral distribution such as the hepatic. Moreover, the absence of a comparable reaction when the irritant is injected into a vein excludes the possibility that the irritant acts upon some distant structure after it has returned to the general circulation. Then the nerve-endings in question must be situated in the artery, in the capillary, or in the perivascular tissues.

Burget and Livingston (1931) noted that a 5 per cent solution of lactic acid did not give origin to pain when injected into the tissues of the thigh of the dog whereas evidences of pain occurred when it was injected into the brachial artery. Similarly, of 8 animals in which we have injected a concentrated sodium-iodide solution directly into the substance of one or another abdominal organ, in only 2 did any reflex effect occur and in these it was delayed and of slight degree. In the same animals subsequent injection of the same irritant into the artery supplying the organ in question resulted in a more immediate and much more marked response. The only tissue in which we have observed regularly occurring reactions to this form of stimulation has been the subcutaneous fatty tissue and in its case the reactions have been of but one-plus intensity whereas injections into the arteries supplying the same area have given four-plus responses. This has led to the conclusion that irritants act upon receptor endings located somewhere in or about the vascular tree.

Odermatt (1922) concluded that the sensitivity concerned is a function of the capillaries, for he found that when all branches of a stem-artery were ligated so as to prevent access from it to arterioles and capillaries the injection of an irritant into the artery did not cause pain. We have repeated this type of experiment. Such evidence lacks conviction when obtained upon the femoral artery where ligation of the numerous branches requires such isolation of the vessel in its bed as to interrupt conceivably its nerve

supply. The left subclavian artery of the cat, however, courses nearly two inches before branching. When ligated proximal to this branching an injection of sodium iodide evoked no response. Preliminary blocking of the femoral arterioles with licopodium spores, moreover, definitely delayed the occurrence of the reaction. These results indicate that the sensory fibers in question terminate in close functional association with the finer arterial branchings.

There has been no generally accepted histological demonstration of afferent fibers which penetrate to the intima of arteries (Wollard, 1926; Kuntz, 1929). Our own microscopic studies of the arterial wall following injection of irritants have shown that the endothelium remained intact and apparently uninjured and the deeper layers of the vessel-wall had not become exposed. These facts may be interpreted as an additional indication that the nerve-endings in question are associated with the finer, thin-coated branches where the irritating solution can rapidly penetrate the vessel-wall.

These afferent nerve-endings associated with the smaller blood-vessels are evidently particularly sensitive to some stimulus of a chemical nature. The irritants used caused no comparably intense stimulation when applied to human skin or when applied to the peritoneum or injected into the tissues of anesthetized animals.

Some veins partake to a minor degree of this same sensibility. The portal vein gave slight reactions in 4 of 9 injections, whereas the inferior cava failed to give a response on repeated trials. The diluting properties of the venous blood-volume may be a factor in this finding, although injection directly into the heart-cavity in one instance gave a four-plus response. Various observers have reported that the injection of irritants into the veins of man is followed by pain (Livingston, 1930).

It is possible that means for other forms of pain-sensibility are present in blood-vessels. Surgeons frequently note pain incidental to mechanical manipulation of arteries. It has been suggested that this sensitivity to trauma is a function of the periarterial plexus (Odermatt, 1922). In our own experience manipulation of arteries has occasionally given rise to reflex activity. But in the cases in which this occurred examination revealed the presence of a visible nerve which had been traumatized along with the artery. In cases in which the blood-vessel had been carefully isolated from all of its accompanying nerve-trunks it was found that neither ligation nor clamping nor stretching nor puncture nor any other form of mechanical manipulation gave signs of reflex disturbance. Many of the peripheral nerve-branches course close to the peripheral arteries. The pain which arises from mechanical trauma to arteries is, in our opinion, properly explained on this basis. Such pain results neither from stimulation of nerve-endings nor from a stimulation in the artery proper, but

arises from physical injury to nerve-fibers which are accidentally included in the manipulation. This interpretation might be extended to explain pain incidental to traction on the mesentery, where, of all tissues affected, the nerve-trunks are most sensitive to tension (Forbes, 1928). Thus we believe that much of the pain caused by many surgical procedures results from trauma to sensory fibers rather than from stimulation to terminal receptors.

We are inclined to minimize the importance of distention of arteries as a mechanism determining the occurrence of painful sensation. We have produced visible distention of the femoral artery in amytalized animals by rapid injection of normal saline without eliciting any appreciable pseudaffective response to indicate that pain-fibers had been stimulated. Knapp (1929) arrived at a similar conclusion. It has been suggested that vascular distention is an important source of pain in inflammations since the pain often follows the rhythm of the pulse and subsides when incision relieves the local swelling and tension (Kuntz, 1929). In this respect it may be pointed out that the increased pressure in inflammatory lesions is obviously both extravascular and intravascular. Incision serves primarily to reduce extravascular pressure, and, were the pain a function of the vascular distention, incision would operate to increase the pain unless there were a secondary adjustment in intravascular tension. It seems more probable that the pain of inflammation arises through some other mechanism, e.g., the mechanical compression of nerve-structures by the increased interstitial pressure.

Spastic contraction of arteries has been generally conceded to be painful. The injection of solutions of barium salts into peripheral arteries gives rise to spasm and to pain. The attacks of vasospasm in Raynaud's disease are accompanied by pain. The pain of angina pectoris is thought to be caused by arterial spasm. Such observations, although convincing to many, are not conclusive proof that the spasm itself is painful. In our experiments the pain arising from injections of barium salts was neither abolished nor appreciably diminished by previous or simultaneous injections of vasodilator substances such as nitroglycerine or acetylcholine. The spasm of Raynaud's disease is a result of exaggerated sympathetic vasoconstrictor activity and an identical form of spasm can be produced by administering adrenalin which stimulates the vasoconstrictor endings. But adrenalin does not cause pain when given by intra-arterial injection or by any other mode of administration. All forms of arterial spasm cause a proportionate ischemia. The spasm produced by adrenalin causes only momentary ischemia whereas that of Raynaud's disease may be maintained for hours until such profound chemical changes occur in peripheral tissues that gangrene results. These chemical changes might plausibly affect nerve-endings. We are not in a position to deny that arterial spasm

is ever of itself painful, but we are inclined to discount its importance in the production of pain relative to that of the chemical changes which take origin from it.

In regard to the pain of ischemia, Lewis, Pickering and Rothschild (1932) have demonstrated that in ischemic contraction of skeletal muscle the pain results from a stimulus which is derived from the accumulation of some factor as a result of physiological processes. We have reported evidence of the existence of afferent fibers subserving pain-sensation that end about the smaller arterial branchings and are particularly sensitive to irritants. It is interesting to note that a 0.1 per cent solution of lactic acid injected into the stem-artery provides strong stimulation of these neurones in anesthetized animals. During conscious states it is possible that more dilute solutions are effective. Lactic acid is known to accumulate in ischemic tissues as a result of anoxidative metabolism. These observations provide an attractive basis for conjecture as to the cause of pain in ischemia. They suggest a simple explanation which is deserving of critical test.

SUMMARY

In animals under sodium-amytal anesthesia the stimulation of afferent neurones subserving pain-sensibility results in reflex motor activity of a pseudaffective nature. When an irritant is injected into an artery there results a strong reflex response which is characterized by movements of the extremities, dilatation of the pupils, changes in the pulse and respiration, and accompanying vocalization.

This form of pain-sensibility is more acute in arteries of the extremities, but is present to a comparable degree in arteries to visceral organs (see p. 260). The pain occurs independent of stretching and of spasm of arterial muscle (see p. 261). It is shown by experiment to result from chemical stimulation of afferent nerve-endings which are located in close relation to the finer arterial branches (p. 262). The chemical properties which serve under natural conditions as an adequate stimulus to these receptors are not discussed at this time.

A study with reference to other possible types of arterial pain-sensibility is reported. It is believed that manipulation of arteries gives rise to pain only as a result of trauma to the accompanying gross nerve-trunks (p. 263). It is suggested that much of the pain which attends many surgical procedures is caused by trauma to nerve-fibers rather than by stimulation of terminal receptors.

Observations are noted which indicate that arterial distention and arterial spasm are of secondary importance in the production of painful sensation (p. 264). In cases in which spastic contraction is accompanied by pain there is present a secondary factor which is a more probable source of the pain. In this connection the irritant property of barium salts may be mentioned and the ischemia which accompanies clinical forms of vasospasm.

The pain which accompanies ischemia is discussed and the efficacy of lactic acid as an irritant stimulus to pain-fibers is noted (p. 265).

It is concluded that there is a numerous group of afferent neurones subserving painful sensation which end in close association with the smaller arterial branchings. These can be stimulated under experimental conditions by the intra-arterial injection of irritants. Under natural conditions it is probable that they function by virtue of a sensitiveness to some chemical stimulus.

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STUDIES ON THE PAIN-SENSIBILITY OF ARTERIES

II. Peripheral Paths of Afferent Neurones from the Arteries of the Extremities and of the Abdominal Viscera

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In the immediately preceding communication (This Journal, p. 259) evidence is presented which indicates the existence of a numerous group of afferent neurones subserving pain-sensibility which end in close functional relation with the finer arterial branches and which are subject to stimulation by irritants. The course of these fibers has been traced by experiment in an attempt to determine the peripheral paths of conduction of impulses arising in arteries and giving origin to painful sensation.

Method. Cats were used in most of the determinations. Sodium-amytal anesthesia was used for the neurectomies and for the intra-arterial injections. The injections were made immediately following the nerve-sections or after a period of several days. A concentrated solution of sodium iodide was the irritant used in the majority of instances (100 grams of the salt added to 100 cc. of water). The reflex reaction to the irritating injection was graded as ++++, +++, ++, +, ? or 0, as defined in the preceding report.

Following complete denervation of an extremity the intra-arterial injection of an irritant still produces a visible local reaction confined to the extremity injected. In the hind limb this local response consists of a slow but sustained extension and stiffening of the extremity, and, in the fore leg, of a gradual flexion of the paw at the wrist joint. This direct stimulation of local structures by the irritant is detected only after all afferent fibers concerned in the reflex response have been divided; otherwise it is obscured by the generalized activity which involves the entire body. In the experiments to be reported care was exercised to distinguish between local and general responses and in cases in which the reaction was confined to the extremity injected the reflex response was graded as absent.

The arteries of the extremities. *Femoral artery.* As control observations, in 39 animals (34 cats and 5 dogs) an irritant was injected into a normally innervated femoral artery. A pseudaffective response occurred in every case and in 36 instances (31 cats and 5 dogs) was of at least three-plus intensity. In 11 cats a similar injection was made after the ipsilateral

lumbar sympathetic chain had been removed in its entirety. Again in every animal a pseudaffective reaction occurred upon injection of the irritant into the artery, and in 10 of the animals it reached a three- or four-plus intensity. In 3 cats an attempt was made to interrupt all of the peripheral spinal nerves to one posterior extremity by section on one side of all branches of the lumbosacral limb-plexus. In each instance the subsequent injection of the femoral artery on that side gave a slight reflex response. However, autopsy revealed that in each animal the lowermost branch to the extremity—the posterior femoral cutaneous nerve—had escaped section. The experiment was then repeated in 5 cats and 2 dogs, particular care being exercised to include this nerve in the section. In these animals injection of an irritant into the femoral artery of the operated side gave rise in no instance to any visible reflex effect. Evidently resection of the lumbar sympathetic trunk did not interrupt any appreciable number of the afferent fibers concerned whereas section of all of the peripheral spinal nerves to the extremity interrupted all of the fibers in question.

In 3 cats the posterior spinal nerve-roots were sectioned on one side, just central to their ganglia, from the 5th lumbar to the 3rd sacral segments inclusive. In each animal the injection of the irritating solution into the femoral artery on the side on which the posterior roots had been divided resulted in no visible reflex response whatever; subsequent injection into the contralateral artery elicited reflex response of four-plus degree. Obviously the fibers in question enter the cord by way of the posterior roots.

Brachial artery. As a control series, in 18 animals (13 cats and 5 dogs) an irritant was injected into a normally innervated brachial artery. In every instance a reflex response occurred and in 16 cases (11 cats and 5 dogs) it was of three- or four-plus intensity. In 2 cats the right cervical sympathetic trunk was removed with its middle and inferior ganglia, following which the right pleural cavity was opened and the stellate and next subjacent thoracic sympathetic ganglia were resected together with the connecting segments of the sympathetic trunk. Injection of the irritating solution into the right brachial artery gave a marked reflex reaction in each animal. Similar injections into the contralateral brachial arteries gave reactions of apparently identical intensity. No appreciable number of the afferent fibers concerned in the reflex from the brachial artery had been interrupted by the operation.

In 3 cats and 1 dog the cords of the right brachial plexus were divided in the axilla, and, as an additional precaution, the lateral divisions of the anterior rami of the 3rd and 4th cervical and of the 2nd and 3rd thoracic nerves were sectioned. In each animal injection of the brachial artery on the side of the operation gave rise to no visible reflex effect whereas injection of the opposite artery gave a four-plus response. The afferent fibers concerned in the reaction had been interrupted in their entirety by division of the appropriate spinal nerves.

In 3 cats the posterior spinal nerve-roots of the right side were sectioned, just central to their ganglia, from the 3rd cervical to the 2nd thoracic segments inclusive, and an injection was then made into the right brachial artery. In none of the 3 animals did any visible reflex effect follow. Subsequent injections into the left brachial arteries gave reactions of four-plus intensity. It was found at postmortem examination that all anterior roots were intact and apparently unharmed. These experiments demonstrate that the afferent fibers concerned in the reflex reaction traverse the posterior roots to reach the cord.

Discussion. Friedrich (1924), Schilf and Stahl (1925) and Abrashanow (1927) have concluded that many of the sensory fibers which are stimulated by injection of irritants into the femoral artery pass centrally along the artery to traverse the lumbar sympathetic trunk on their way to the spinal cord. Dennig (1924, 1925), Brjussowa and Lebedenko (1929), and Burget and Livingston (1931) have reported experiments in which section of the posterior spinal nerve-roots has proved more effective in abolishing such pain than has resection of the sympathetic trunk or division of the femoral artery.

The experiments reported at this time demonstrate beyond doubt that the pain-fibers which are stimulated by injection of irritants into the brachial or into the femoral artery pass to the spinal cord by way of the peripheral spinal nerves and their posterior roots. It is certain that no appreciable number of the fibers in question traverse either the sympathetic chain or an anterior spinal nerve-root. Their peripheral course is analogous to that of the posterior-root fibers which subserve cutaneous sensations.

The conclusions of Schilf and Stahl and of Abrashanow are, in our opinion, unwarranted. These observers worked with animals in which only the femoral and sciatic spinal nerves had been sectioned. The hind limb of such an animal is in no way deprived of its entire somatic innervation. Experiments which we have reported demonstrate the presence of afferent fibers which subserve the pain-sensibility of the femoral arterial tree in other peripheral branches of the lumbosacral plexus, e.g., in the posterior femoral cutaneous nerve. The observers mentioned performed their experiments upon the dog and used a 50 per cent solution of lactic acid as the irritant. In order to be certain of our conclusions we repeated the experiments upon the femoral artery of the dog and used lactic acid for the injection. Section of all branches of the lumbosacral plexus completely abolished the reflex response to lactic acid in the dog just as it had abolished the response to sodium iodide in the cat.

We have been unable to confirm Abrashanow's report that after section of the femoral and sciatic nerves in the dog a part of the residual reflex reaction to the intra-arterial injection of irritants is abolished if the femoral

artery is divided proximal to the site of injection. In our experiments the injection of 50 per cent lactic acid into the divided artery gave as marked a reflex reaction as did injection of the contralateral vessel. Abrashanow used ether as the anesthetic agent. In our experience anesthesia under light etherization is apt to vary so greatly in depth from moment to moment as to invalidate the comparison of reflex effects which are elicited in temporal series. Under the more constant anesthetic action of sodium amytal we could obtain no evidence that a part of the fibers in question ascend in the coat of the vessel.

THE ARTERIES TO THE ABDOMINAL VISCERA. These experiments were performed upon cats. A midline laparotomy was done under sodium-amytal anesthesia and the artery to be studied was injected from the exposure thus afforded. When a neurectomy preceded injection of the artery an autopsy was performed to determine whether the nerves had been sectioned as intended. As reported in a previous communication, arteries to the abdominal viscera are somewhat less acutely sensitive to irritants than are arteries to somatic tissues.

Hepatic artery. As a control series, of 22 normal cats in which an irritant was injected into the hepatic artery, in 17 a definite pseudaffective reaction followed and in 8 it was of three-plus or four-plus intensity.

In 8 animals both major splanchnic nerves and both lumbar sympathetic trunks were divided at the level of the diaphragm. Injection of an irritant into the hepatic artery at an interval varying from a few minutes to one week after the nerves had been sectioned gave rise to no sign of reflex reaction in any animal of the 8. Hence all afferent fibers concerned in the response to injection of this artery ascend either the lumbar sympathetic trunk or the major splanchnic nerve to enter the cord in the thoracic region.

In 5 animals in which both major splanchnic nerves were divided at the level of the diaphragm but the lumbar sympathetic chains left untouched so that impulses might still ascend in the chains from lumbar to thoracic levels, subsequent injection of an irritant into the hepatic artery gave rise to no reflex effect. These cases prove that all of the fibers in question ascend to the thorax by way of the major splanchnic nerves and that none of them enter the lumbar sympathetic trunk by way of the more posterior or minor splanchnic branches.

In one animal the left major splanchnic nerve was sectioned at the diaphragm. In this animal the injection of the hepatic artery gave a reflex response of four-plus intensity, demonstrating that many of the fibers in question are contained in the right major splanchnic. In 5 animals the right major splanchnic nerve was sectioned. Subsequent injection of the hepatic artery gave no reaction in one instance, a one-plus reaction in 3 instances, and a three-plus response in the remaining case. These experiments indicate that a certain number of the fibers from the hepatic artery ascend in the left major splanchnic.

In summary, all afferent fibers subserving pain-sensation and stimulated by the injection of an irritant into the hepatic artery ascend in one or other of the two major splanchnic nerves to enter the spinal cord at some dorsal segment. Judged by the response, most of the fibers in question seem to

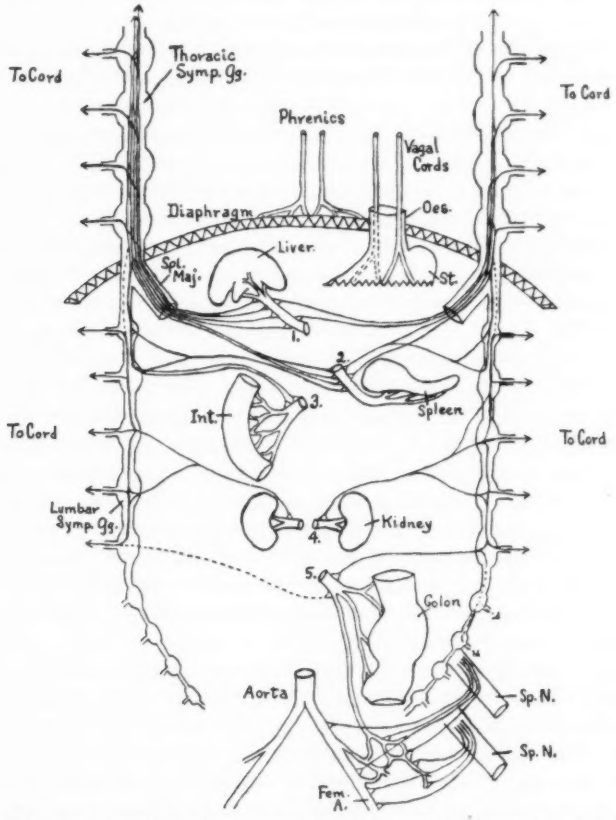

Fig. 1. Diagram of peripheral paths of pain-fibers supplying arteries of abdominal viscera of cat (see text). Dotted lines indicate additional paths which could not be excluded.

Arteries: *1*—hepatic; *2*—splenic; *3*—superior mesenteric; *4*—renal; *5*—inferior mesenteric.

ascend the right major splanchnic nerve, but a definite number are contained in the left major splanchnic (see fig. 1).

Splenic artery. Injection of an irritant into the splenic artery gave an unmistakable pseudaffective reaction in 10 of 12 normal cats. The reac-

tion was quite marked in 4 animals and of but moderate or slight intensity in the remaining 6.

Injection failed to give any reaction whatever in 11 animals in which both major splanchnic nerves and both lumbar sympathetic chains had been sectioned at the diaphragm. Hence all of the fibers which supply the splenic artery enter the cord in the thoracic region.

In 1 animal both major splanchnics were cut but the lumbar sympathetic chains were left unharmed. In this animal injection of the splenic artery gave a two-plus response. In 2 other instances the major splanchnics were left intact but both lumbar sympathetic chains were resected. In each of these animals injection of the splenic artery resulted in a one-plus reaction. Therefore, some of the fibers from the splenic artery ascend in the sympathetic trunks after entering them below the level of the major splanchnics, while others of them ascend in the major splanchnic trunks.

In 2 animals the right major splanchnic and the right lumbar sympathetic trunk were divided at the diaphragm whereas the nerves of the left side were left intact. In 1 of these animals the injection resulted in a response of one-plus intensity and in the other animal a reflex effect was questionable. In 1 animal the left major splanchnic and left lumbar sympathetic trunk were resected and in it the injection of an irritant into the splenic artery was followed by a three-plus response. These cases indicate that the fibers from the splenic artery ascend on both right and left sides although probably a majority of them ascend on the right.

In summary, the afferent fibers subserving the pain arising from irritating injections into the splenic artery enter the spinal cord in the thoracic region. They ascend from the abdomen both in the sympathetic trunks and in their major splanchnic branches, and probably more of them ascend on the right than on the left (see fig. 1).

Superior mesenteric artery. This artery was injected with an irritant in 12 normal animals. In 9 of them a reflex response occurred but in only 1 did it exceed two-plus in intensity. The superior mesenteric artery is less sensitive to irritants than are the hepatic and splenic arteries.

In 10 animals in which both major splanchnics and both sympathetic trunks were interrupted at the diaphragm the injection of an irritant into this artery gave rise to no visible response. A similar absence of reaction occurred in 2 animals in which both lumbar chains had been resected without section of the major splanchnics. In 4 animals in which the lumbar chains were intact but the major splanchnics interrupted the irritant gave rise to a reaction in 2 cases. In 3 animals there was no response after the right chain had been resected and right major splanchnic divided. On the other hand in 1 animal in which a corresponding operation had been performed on the left side only, the injection gave rise to a four-plus response.

It is concluded that the majority of the afferent fibers supplying the superior mesenteric artery enter the right lumbar sympathetic chain posterior to the major splanchnic nerve and ascend the sympathetic trunk to enter the cord in its dorsal segments (see fig. 1).

Renal arteries. One or other of the renal arteries was injected in each of 20 controls but in only 12 instances did a pseudaffective reaction result and in no instance did it exceed a two-plus intensity. The renal artery is less constantly sensitive to irritants than is any other abdominal artery we have tested.

The renal artery was injected in each of 7 cases in which both major splanchnics and both lumbar chains had been divided at the diaphragm. In 4 of these cases a reaction occurred and in 3 it reached a two-plus intensity. In another animal in which both major splanchnics had been sectioned but the sympathetic chains left intact the renal artery gave a two-plus reaction. These results serve to show that the fibers supplying each renal artery enter the lumbar sympathetic chain of that side and from it pass into a lumbar segment of the spinal cord (see fig. 1). This finding is surprising in view of the frequency with which renal disease causes referred pain which is localized in thoracic segmental areas (Head, 1893).

In 20 other instances a renal artery was injected following one or another combination of neurectomies but in none of these cases did a reflex effect occur. The sensitivity to irritants is evidently very slight in the renal arteries of many animals. In no animal, however, have we seen it in evidence after resection of the lumbar sympathetic trunks.

The inferior mesenteric artery gave a greater percentage of strong reactions than did any other lower abdominal artery. In each of 20 animals a definite pseudaffective response resulted, and in 5 of these cases was of three-plus degree. No combination of abdominal neurectomies was found that would abolish this reaction in the case of the inferior mesenteric artery. Both lumbar sympathetic chains may be resected and both major splanchnic nerves sectioned without diminution of the intensity of the response, although the latent period may be prolonged. Probably this peculiarity of the artery is a result of its anastomotic connections by way of which the irritant passes to arterial channels in the regions of the hip and thigh. These regions are supplied, as shown in a previous section, by fibers which ascend the peripheral spinal nerve-trunks without traversing the sympathetic chain.

Discussion. It is interesting to note the importance of the sympathetic trunks and their splanchnic branches in relation to the pain-sensibility of the abdominal organs. In four animals in which both vagal cords had been severed at the cardia the injection of irritants into the arteries to the abdominal viscera gave rise to just as strong reflex reactions as were caused by similar injections in normal animals. When the sympathetic

and splanchnic elements were interrupted, however, the arteries became anesthetic although the vagi and the phrenic nerves had not been disturbed. In several animals in which all sympathetic and splanchnic paths from the abdomen had been previously interrupted a laparotomy was done under quite light sodium-amytal anesthesia. There was constant reflex activity during the incision of the skin and again during the cutting of the submuscular layer (the posterior sheath of the rectus fused with the parietal peritoneum). But when this work was completed in the domain of the intercostal nerves and the abdomen opened it was found that the abdominal contents could be traumatized freely without causing the slightest reflex effect.

Bradford Cannon (1932) obtained interesting results in experiments in which a stimulus was applied to one or another visceral nerve in the unanesthetized cat by means of electrodes buried in contact with the nerve during a preparatory operation. Stimulation of the vagus trunk seemed to be disturbing only because it resulted in difficulties in respiration. But as soon as the slightest stimulus was applied to electrodes set on a splanchnic nerve the animal was at once restless.

The afferent neurones which are contained in the vagus and phrenic nerves and which are concerned with referred pain can hardly, in the light of recent interpretations of the mechanism of referred pain (Weiss and Davis, 1928), be classed as true pain-fibers. Moreover, the majority of the afferent fibers contained in these nerves carry impulses which do not, even indirectly, give rise to pain. It is the sympathetic trunks and their splanchnic branches which contain the true pain-neurones supplying the abdominal organs.

It is evident that an abnormal process in a visceral area may result in stimulation of afferent fibers of several orders and of varied peripheral paths. Walton, Moore and Graham (1931) demonstrated that the afferent fibers which operate to produce the vomiting of peritonitis pass centrally both in the sympathetic trunks and in the vagal cords. The results given above, on the other hand, indicate that the pain of peritonitis is a function of sensory fibers which follow, without exception, a sympathetic (or splanchnic) path. Weiss and Davis (1928) found that, although anesthetization of the area of skin to which the pain of biliary colic is referred is effective in abolishing the pain, it is not effective in relieving the vomiting, and, similarly, Schrager and Ivy (1928) have reported that the vomiting which accompanies distention of the biliary ducts in the dog may be relieved by vagotomy but distress persists until the right splanchnic nerves are cut.

SUMMARY

The injection of an irritating solution into an artery results in stimulation of afferent fibers which subserve arterial pain-sensibility. In amytal-

ized animals this results in characteristic reflex motor activities of a quasi-emotional nature. The peripheral anatomical paths which these fibers follow have been traced in experiments determining the presence or absence of this reflex reaction after section of one or another peripheral nerve.

The fibers of this type which supply the femoral and brachial arteries have a peripheral course identical to that of the posterior-root fibers which subserve cutaneous sensibility. They pass centrally in the peripheral branches of the spinal nerves and enter the spinal cord by way of the posterior roots (see p. 267). No significant number of them course long distances along the arteries, or traverse the sympathetic chain, or enter the cord by an anterior root.

In contrast with these fibers to the extremities, the fibers which are stimulated by injection of irritants into the hepatic, the splenic, and the superior mesenteric arteries are shown by experiment to ascend either the major splanchnic nerves or the sympathetic chains and to enter the spinal cord in the thoracic region (see p. 271, fig. 1). On the other hand, the fibers of similar function which supply the renal arteries enter the spinal cord in the lumbar region after having traversed a minor splanchnic nerve and one of the lumbar sympathetic chains (p. 273).

Experiments are reported (p. 273) which illustrate the importance of the sensory fibers contained in the sympathetic and splanchnic trunks as regards the pain-sensibility of the abdominal organs. Afferent fibers contained in the vagal cords and in the phrenic nerves subserve varied and important functions, but seldom conduct impulses which give rise to painful sensation. In respect to their afferent components, the sympathetic trunks and their splanchnic branches are the true pain nerves of the abdomen.

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A QUANTITATIVE STUDY OF THE CHANGES IN POWER DURING MUSCULAR CONTRACTION

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The term power is used in this paper in its technical signification which means the rate at which work is done. In order to measure the power put forth by a contracting muscle, it is required to know the force exerted during a sufficiently small interval of time together with the distance through which the muscle shortens during the same time interval. From the measurement of force for each corresponding length of muscle, the work done during the same time interval can be computed. The power expended is the quotient of the work done divided by the time taken to do it. The figure, thus obtained, expresses, in terms of the units employed, the rate at which the muscle does its work. Power can also be expressed as the product of force and the speed of contraction. Since considerable use will be made of this relationship farther on in this paper, it will facilitate the exposition of our results if the derivation of power in terms of force and velocity is introduced here.

Since the method employed in these experiments relates increments of force to increments of length, the relations of power, p; work, w; force, f; time, t; distance, s; and velocity, v are expressed in terms of differential calculus. As already defined, power is the rate of doing work, or,

$$p = \frac{dw}{dt} \tag{1}$$

Work, by definition is the product of force and distance.

$$dw = fds \tag{2}$$

Velocity is the ratio of space to time or $\frac{ds}{dt}$. Substitute fds for dw in equation (1) and obtain,

$$p = \frac{fds}{dt} = fv \tag{3}$$

[1] Acknowledgment is gratefully made to Prof. R. H. Stetson of Oberlin College for placing at our disposal the resources of the Psychological Laboratory in which this work was done and also for much helpful criticism. We are, likewise, deeply

A special condition arises when work is done under constant power. In this case, force and velocity vary inversely and the curve relating them is a hyperbola. Therefore, as velocity increases, force must necessarily decrease. We have calculated the power in our experiments from the force exerted by the muscle during a twitch and a tetanus and the speed with which it contracts during each ninetieth of a second, with the result that the power is found to be approximately constant after the muscle has attained maximal tension until relaxation begins. The well known fact that the force of a muscle decreases as the speed of contraction increases, is, therefore, interpreted to mean that striated muscle, during a part of its cycle, does its work under approximately constant power. This interpretation has been set forth in a previous paper by Stevens and Snodgrass (1).

The present paper is concerned with the quantitative determination of the speed of shortening of the muscle, the force developed, the work done and power expended during each 0.011 second of the contraction cycle. With the technique employed in these experiments both tension and length are allowed to vary concomitantly during the same contraction against an inertia system. By means of optical recording and a timing device, the change of tension and change of length for each time interval of 0.011 second or 0.001 second are determined. The instantaneous tensions, therefore, with the instantaneous lengths are thus recorded independently of each other for time intervals up to one-thousandth of a second. By means of this technique it is possible to know accurately the entire range of tension changes and corresponding length changes throughout the same contraction cycle.

A somewhat similar method has been employed by Hill (2) and by Lupton (3) using inertia discs (pulleys) and also by Fenn, Brody and Petrilli (4) each using intact human muscles. Fenn's method was essentially that used in this paper since he inscribed time in 0.01 second on his kymograph record. The usefulness of his results, however, was limited by the short excursion of the limb which was permitted. In their experiments the independent registration of the changes of length of the muscles was not possible. In our experiments in which the cat was used, it seemed desirable, for three reasons, to record the changes in the length of the muscle independently of the inertia system by which the tension of the muscle was measured: *a*, in order to determine the moment at which relaxation begins; *b*, to be able to measure the velocity of the contractile

indebted to Prof. L. W. Taylor of the department of Physics for the use of apparatus and for an analysis of the physics of the problem. Professor Taylor also pointed out to the senior author that motion under constant power implies an inverse relationship between force and velocity. It was this suggestion that led to the search for evidences of constant power in muscular contraction.

process in the muscle itself independently of the velocity of the inertia system, upon which the muscle acts, on the assumption that the velocity of the muscle may be, at some points, different from the velocity of the inertia mass; this assumption has been justified as may be seen by reference to figure 4, in which the velocity of the inertia disc and the velocity of the muscle are plotted against the same time intervals; *c*, to be able to compute the power of the muscle or the rate at which it does work.

EXPERIMENTAL PROCEDURE. The gastrocnemius muscle of the decerebrate cat was used in these experiments. The animal was mounted in the manner described by Stevens and Karrer (5). The muscle, severed at the tendon and dissected free except for its attachments at the knee was supported on a glass plate in the horizontal position under an initial tension of 225 grams which was removed at the moment of stimulation. It was kept well moistened with mammalian Ringer's solution and was warmed by an electric lamp placed beneath it. The femur of the leg used was exposed and clamped securely to prevent any yielding at the time of stimulation. The muscle was attached to the inertia disc by a flexible steel cable and a hook through the tendon around the tuber calcanei. As these technical details are important in a quantitative experiment of this sort because of errors which may be introduced by them, a few words may be devoted to this subject. The ideal wire of theoretical mechanics is flexible which means that no force is required to bend it; it is inextensible, which means that the velocity at the two ends is equal; it is weightless which means that the force at the two ends is equal. The actual wire (fig. 1, *R*) used was made up of 7 strands of no. 35 steel piano wire soldered together at the ends while under equal tension and at one additional point along its length so as to form a flat cable which lay on the bottom of the flat groove without shifting. This wire did not meet the second requirement of theoretical mechanics. This error, however, was rendered ineffective because we measured the velocity both at the muscle end and at the disc end of the wire.

The apparatus, a diagram of which is shown in figure 1, consists of three essential parts; the inertia system, *B;* the optical system, *D;* time-disc, *E*, and camera, *C*, and the stimulating mechanism, *G*.

The inertia system (fig. 1, *B*) consists of eleven metal discs 17.8 cm. in diameter and weighing 13.6 kgm. which can be superposed and bolted together to give greater or less inertia. A circular hole 3.8 cm. in diameter was cut from the center of each disc. Certain of the discs were made of lead and others of sheet steel. These discs were attached to a steel rod which served as a vertical axis and suspended on a steel wire 0.84 mm. in diameter and 22 cm. long (fig. 1, *I*). The lower end of the axis was hollowed out and rested lightly on a single, steel ball (fig. 1, *J*). The moment of inertia of the composite disc was 495,000 gm.-cm^2. The lower disc was

grooved to serve as a pulley for the traction wire. The radius of this disc to the bottom of the flat groove is 8.65 cm.

Time in thousanths of a second was imprinted on the photographic record by means of a slotted, metal disc (fig. 1, *E*) which was rotated by a synchronous motor, *F*, at a speed of 30 revolutions per second so as to cut a thin beam of light from a 500 watt lamp suitably focussed on the camera aperture. There were 33 radial slots cut in the disc of which every eleventh was about twice the width of the others. The actual length of time be-

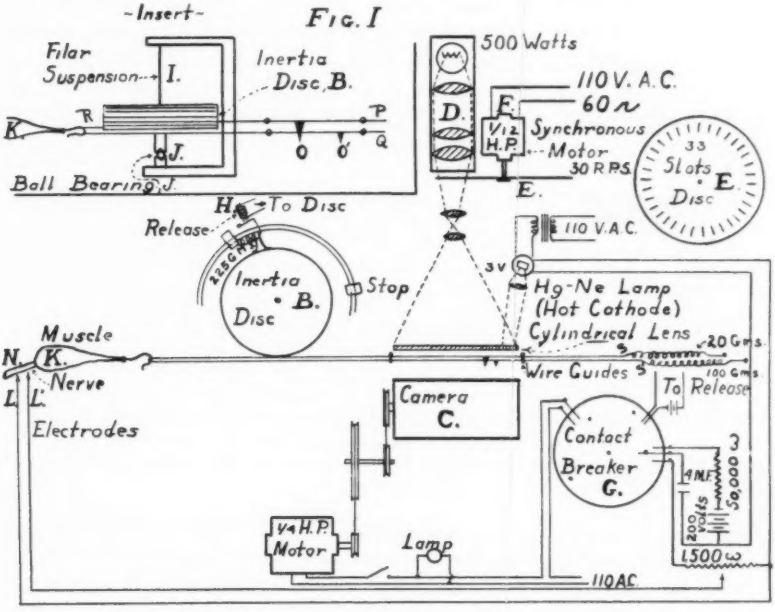


Fig. 1. Diagram of apparatus. See text for description

tween successive slots is therefore 1/990 of a second giving an error of ten in a thousand or 1 per cent. The interval between the heavier lines is 1/90 second or about 0.011 second.

The muscle was maximally stimulated by platinum electrodes through the tibial nerve by the discharge of a 4 microfarad condenser in the case of the twitch responses. The tetanizing stimulus was a rectified 60 cycle alternating current consisting of 120 uniform pulses per second. A Porter inductorium was also used in some of the earlier records. The duration of the condenser discharge was about 25 sigma. Some variation in duration of the stimulus resulted from accidental variations in the switch. The rotating contact breaker, by means of switches appropriately placed (fig.

1, *G*) removed from the muscle the initial tension of 225 grams (fig. 1, *H*), started the motor which turned the photographic paper in the camera (fig. 1, *C*), stimulated the muscle and finally stopped the camera motor. The stimulus was recorded on the photographic paper by a mercury neon lamp in the circuit.

The photographic record of an experiment, an example of which is given in figure 2, shows the vertical time lines which are one-thousandth of a second (1/990) apart with the heavier lines every ninetieth of a second apart. At the upper left hand corner is the blackened area which marks the duration of the stimulus registered by the flash of the mercury-neon lamp. The three horizontal white lines on the record, reading from top to bottom, are the upper base line, the shortening of the muscle, and the displacement of the disc. These lines are drawn by aluminum markers (fig. 1, insert *O* and *O'*) which cast a shadow on the photographic paper.

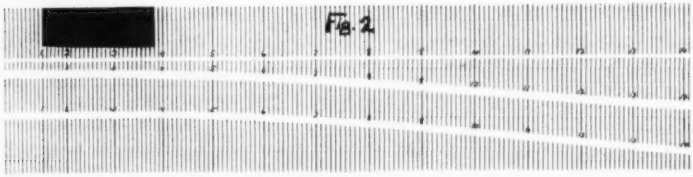


Fig. 2. Photographic record of an experiment. Read from left to right. Heavy vertical lines indicate time in 0.011 second; lighter vertical lines indicate time in 0.001 second. Upper white line is the base line; middle white line is the shortening of the muscle; lower white line is the acceleration of the disc. The blackened area at the upper left hand corner is the stimulus.

The two base lines (only one is shown in fig. 2) are made by wires fastened across the slit in the camera. One fine steel wire (fig. 1, *Q*) carrying its marker, *O'*, is soldered on to the hook which is attached to the tendon of the muscle. The other wire (fig. 1, *P*) carrying its marker, *O*, is attached to the disc. The muscle marker measures the change of length of the muscle without magnification by a direct pull with no mechanical source of error other than stretch of the tendon. This wire is kept taut by a spring (fig. 1, *S'*) of 100 grams tension so that the moment of relaxation will be marked on the record. The wire carrying the disc marker is kept taut by a rubber band (fig. 1, *S*) 15 cm. long with a tension of 20 grams which changes very little with the displacement of the disc. Measurements of the muscle and disc displacements were made by means of a Gaertner comparator reading to one one-thousandth of a centimeter and a reading microscope magnifying the record about 40 times. This method of reading the records to obtain the slopes of the curves is very laborious, but is accurate to within ± 0.001 cm. The earlier records were read for each 0.001 second for a duration of 200 sigma. It was found, however,

that 0.011 second was sufficient for the purposes of these experiments with a duration of about 120 sigma.

The velocity of the disc was determined by taking the differences between two adjacent displacements and dividing by the time interval between them. The acceleration was determined by taking the difference between two adjacent velocities and dividing by the time interval between

TABLE 1

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
TIME	MUSCLE SHORTENING	CHANGE OF LENGTH OF MUSCLE	MUSCLE VELOCITY	ANGULAR VELOCITY OF DISC	ANGULAR ACCELERATION OF DISC	FORCE	WORK	POWER $F \times v$
seconds	*cm.*	*cm./0.011 second*	*cm./second*	*radians/second*	*radians/second²*	*kgm.*	*kgm.-cm.*	
0	0	0	0	0	0	0	0	0
0.0027			(0.065)					
0.0055			0.13	0.014				
0.011	0.0015	0.0015	(1.13)		2.4	0.142	0.0018	0 16
0.0165			2.14	0.040				
0.022	0.0251	0.0236	(3.24)		18.9	1.103	0.0394	3 54
0.0275			4.34	0.250				
0.033	0.0729	0.0478	(5.12)		35.0	2.050	0.1153	10 39
0.0385			5.90	0.639				
0.044	0.1377	0.0648	(7.27)		33.2	1.930	0.1541	13 88
0.0495			8.64	1.007				
0.056	0.2328	0.0951	(9.75)		28.2	1.650	0.1741	15 68
0.0615			10.87	1.320				
0.067	0.3521	0.1193	(11.79)		22.1	1.288	0.1669	15 02
0.0725			12.71	1.566				
0.078	0.4920	0.1399	(13.37)		19.0	1.112	0.1638	14 72
0.0835			14.04	1.778				
0.089	0.6464	0.1547	(14.42)		10.1	0.591	0.0940	8 45
0.0945			14.81	1.890				
0.100	0.8094	0.1630	(14.72)		8.1	0.474	0.0769	6 92
0.1055			14.63	1.980				
0.111	0.9704	0.1610	(13.87)		0.4	0.024	0.0358	3 23
0.1165			13.12	1.985			1,022,100 ergs.	
0.122	1.115	0.1446						
0.1275								
0.133								

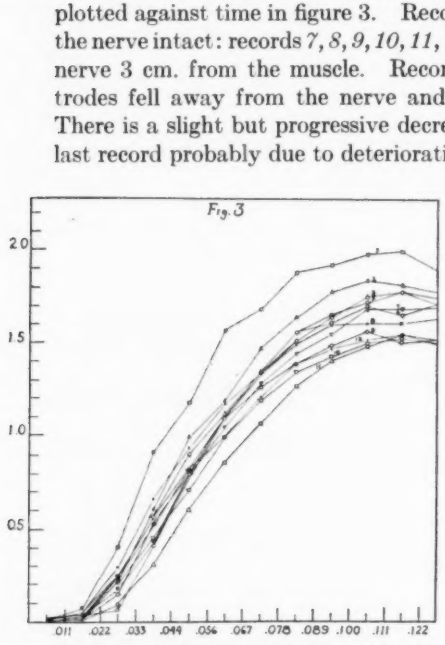
them. By these operations the first derivative (velocity) and the second derivative (acceleration) are obtained.

Force in dynes is calculated by dividing the torque by the radius (8.65 cm.) of the inertia disc. The torque is equal to the product of moment of inertia and the angular acceleration. To express the force in kilograms, the force in dynes is divided by 980,000.

The results which are presented in table 1 are the averages of eleven

records obtained from the same animal, cat 481, during the same experiment. These results are selected as representative of a series in which twenty animals were used.

The individual disc velocity curves in rad./sec. of the eleven records are plotted against time in figure 3. Records *1*, *2*, *3*, *5*, and *6* were made with the nerve intact: records *7*, *8*, *9*, *10*, *11*, *12* were made after severing the tibial nerve 3 cm. from the muscle. Record *4* was excluded because the electrodes fell away from the nerve and the stimulation was not maximal. There is a slight but progressive decrease in velocity from the first to the last record probably due to deterioration of the animal during the 1½ hour



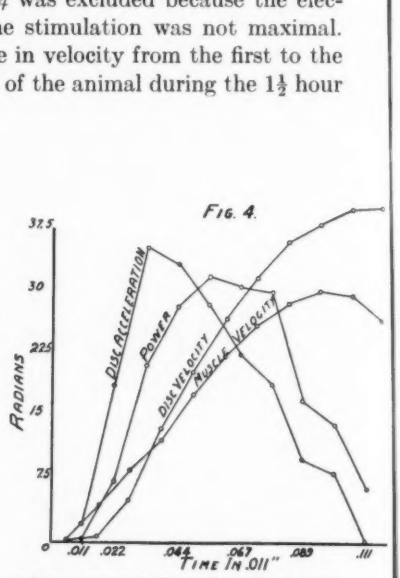


Fig. 3. The angular velocity of the disc in radians/second plotted against time for the eleven records obtained from cat 481. To show the range of variation of the original data.

Fig. 4. The angular velocity of the disc, linear velocity of the muscle and angular acceleration of the disc are plotted against time. Power or the rate at which work is done is also plotted against time.

period of the experiment. An interval of seven minutes separated one record from the next. Section of the nerve does not greatly alter the response. The average disc velocity in centimeters per second for each 0.011 second together with the mean variation of the average and the probable error of the mean is given in table 2. The graphs of figure 3 and the numbers in table 2 indicate the range of variation in the method. A part of this variation is due to the fact that the disc did not start at the same instant in successive records.

The average measurements of these records are shown in table 1 for each 0.0055 second (column 1). Since the velocity measured is the average

velocity over the time interval of 0.011 second, the figure for velocity in the table is placed midway between the beginning and the end of the interval. Since acceleration is also an average over an equal time interval, the figure for acceleration is placed between the figures for velocity. In plotting the velocities and accelerations against time in figure 4, the same relations were maintained. The rate of change of length of the muscle for each 0.011 second is shown in column 3. The total shortening attained up to the eleventh time interval is given in column 2. Muscle velocity in centimeters per second is given in the fourth column of the table. These velocities are obtained by dividing the figures in column 3 by 0.011 second. The figures in brackets in column 4 are the average muscle velocities which correspond in time to the forces in column 7.

The angular velocities of the disc and disc accelerations in radians per second are given in the fifth and sixth columns. Force in kilograms (col-

TABLE 2

	TIME INTERVAL													
	0.011 second	0.022 second	0.033 second	0.044 second	0.056 second	0.067 second	0.078 second	0.089 second	0.100 second	0.111 second	0.122 second	0.133 second	0.144 second	0.156 second
Average disc velocities, cm/sec.	0.138	0.390	2.41	6.15	9.67	12.7	15.04	17.08	18.16	19.00	19.09	18.80	18.57	18.7
Mean variation	0.07	0.17	0.83	1.24	1.29	1.23	1.20	1.38	1.31	1.33	1.36	1.19	1.16	1.03
Per cent of average velocity	50	43.6	34	20.1	13.3	9.7	8.0	8.1	7.2	7.0	7.15	5.8	6.2	5.0
PE of mean	0.018	0.045	0.224	0.335	0.349	0.331	0.324	0.373	0.352	0.359	0.367	0.321	0.314	0.278

umn 7) is derived from acceleration (column 6) by the method just outlined. The work done for each interval of 0.011 second maybe computed either by multiplying force (column 7) by the corresponding change of length (column 3) or by multiplying power by the time interval.

A graphic integration of the force-length area per unit time is shown in the dotted step-wise graph of figure 5. The sum total of work integrated by adding together the rectangles for each time interval in column 8 is 1,022,000 ergs which agrees within 4.6 per cent with the value of 976,000 ergs found by computing the kinetic energy of the disc. From table 1 which contains the basic data of these experiments, the average disc velocity and average acceleration curves, both unsmoothed, are plotted against time in figure 4. The curve of muscle velocity, in cm./sec. plotted on the same coördinates is also shown. In these graphs, the same scale is made to serve for the four variables by multiplying in each case by a factor. The factor for acceleration is 1; for the velocity of the disc., $\frac{1}{20}$;

for the muscle velocity, $\frac{1}{2}$; and for the power $\frac{1}{2}$. Two features of the velocity curves deserve mention. The velocity of the muscle is at first greater than that of the disc, indicating some stretch in the wire connection with the disc. Then, as the disc speeds up, its velocity exceeds that of the muscle. A part of the increased speed of the disc is due to the elastic recoil of the stretched wire. The maximal speed of the muscle is reached between the 8th and 9th time interval after stimulation. The maximum of the disc is reached 0.011 second later. The muscle velocity then falls off and the disc, because of its greater momentum, loses speed much less slowly. The force of the muscle attains a maximum at 30 sigma after stimulation and goes to zero at about 100 sigma after stimulation.

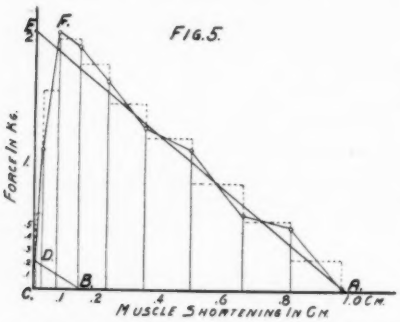


Fig. 5. The tension length diagram of the eleven records of C 481 averaged together. The triangle B C D represents the work done on the muscle by the initial, passive, extension. The rectangles represent the work done in successive time intervals.

The tension-length diagram, figure 5, is drawn by plotting the shortening of the muscle (column 2, table 1) against force in kilograms (column 7, table 1). Taking 0.985 cm., the amount of shortening of the muscle when force went to zero, as the base of a triangle and 2,050 kgm. the maximal force developed as the apex, A B C E will represent the work done by an elastic body shortening through 0.985 cm. The actual work done by the muscle is shown by the figure, A B D F. The triangle B C D represents the work done on the muscle by the initial, passive tension of 225 grams. The area of this triangle, B C D, was calculated from data obtained from an "instrumental" run (cat 473, record 9) in which the disc was released by the contact breaker without stimulation of the muscle. The kinetic energy of the disc due to the sudden release of the stretched muscle is equal to one-half of the product of the moment of inertia and the square of the angular velocity. The moment of inertia of the disc, in this experiment was 290,000 gm.-cm^2. The angular velocity of the disc measured in the same manner as that described above, was found to be 0.304 radian per second. After squaring this velocity and multiplying it into the moment of inertia and dividing by two, the result obtained is 13,400 ergs. To convert this figure into kgm.-cm. of work it is divided by 980,000 obtaining the result of 0.0136 kgm.-cm. which is the product of a force and a distance. To find the distance through which the muscle is stretched by

225 grams, 0.0136 kgm.-cm. is divided by 0.225 kgm. or the tension of the spring. The result is 0.0604 cm. which is multiplied by 2 because the initial force was zero. The length to which the muscle was stretched by the initial force of 225 grams is, therefore, 0.1208 cm. This figure is base of the triangle B C D in figure 5. This work B C D should be deducted from the actual work done by the muscle. It is evident from figure 5 that the work done by the muscle approximates that of an elastic body shortening through a distance equal to that through which the muscle actually shortened in doing work on the disc.

The work done by the muscle may be computed in two ways from the measurements recorded in table 2, depending on whether the kinetic energy of the disc is employed or the product of force and muscle shortening. In the former method,

$$W = \frac{I\,\omega^2}{2}. \qquad I = 495{,}000$$

The maximal angular velocity, ω, is 1.985 rad./sec. The computation is, therefore,

$$\frac{495{,}000 \times 3.94}{2} = 976{,}000 \text{ ergs.}$$

In the latter method $W = \int f ds$. An approximate integration is obtained by adding together the elements of work for each time interval in column 8, table 1. To obtain the total work done by the muscle, the work done on the springs of the muscle marker and disc marker should be added to the figures calculated from the work done on the disc. Since the total force of the springs was 120 grams plus 20 grams allowed for friction, and the distance through which it acted was 0.985 cm., the work done is $140 \times 980 \times 0.985$ cm. = 135,100 ergs. The work done on the muscle in the initial stretching must be substracted from the work calculated above. This work has been shown to be 13,400 ergs. With these corrections, the work done, calculated by the two methods is 1,097,000 ergs and 1,143,700 ergs, respectively.

The ratio, $\frac{W}{Tl}$ may be calculated from these data. W (work) is derived from the figure 1,097,000 ergs by dividing it by 980,000 to find the equivalent work in kilogram-centimeters. $W = 1.120$ kgm.-cm. $T = 2{,}050$ kgm., the maximal tension. $L = 9.9$ cm. which is the length of the muscle under a tension of 225 grams. Hence $\frac{1.120}{20.29} = 0.0489$ which is a fair approximation to the 0.04 found by Doi (6).

The curve relating the force developed to the velocity of muscle shorten-

ing is plotted in figure 6 from the data in table 1 (columns 4 and 7). It is apparent that as the speed of contraction increases, once the muscle has developed maximal tension, the force diminishes. This inverse relation between force and speed of contraction appears in the results of all workers who have made the necessary measurements. It has been explained by A. V. Hill (7) as due to a viscous-elastic factor which impedes the speed of shortening. After referring to visco-elasticity as one of the "major factors in the dynamics of muscular activity," Hill says, "Expressed in a sentence this factor simply is that the faster an active muscle shortens the less force it can exert, the less work it can do." While admitting that some of the loss of force with speed is due to internal, frictional resistance, Fenn (8) suggests that there is another factor based upon the fact that a shortening muscle expends more energy than when it is being stretched or when it is contracting isometrically. "The added necessity for redeveloping tension during shortening and the delay in the chemical reactions necessary to supply energy for this process is one of the factors which limit the tension in moving muscles."

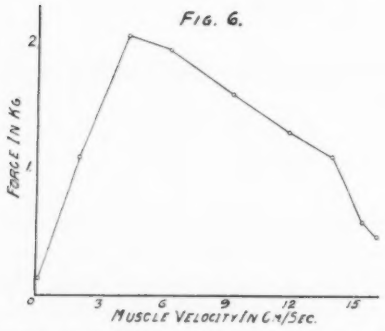


Fig. 6. The force-velocity curve of the eleven records of C 481 averaged together.

There is, as already stated, an alternative interpretation of a purely mechanical sort which may now be further considered. This interpretation depends upon the fact that in motion under constant power an inverse relation exists between force and velocity. L. W. Taylor (9) in discussing motion under constant power says, "Most of our engines of transportation work at approximately constant power. An automobile, thanks to the gear-shift, develops substantially the same power when starting that it does on the road. Street-cars develop nearly the same power when pulling slowly up a hill as when running at normal speed with a heavy load. Everywhere we see examples of motion under constant power. Yet it is safe to assert that the reader has never encountered a discussion of the laws of motion under constant power.

"Physics text-books are full of examples of motion under a constant force, of which simple falling is the best example. But motion under constant force is a very different thing than motion under constant power. For example, under constant power, the acceleration becomes smaller as velocity increases, whereas under constant force, the acceleration is always

the same, whether the velocity is large or small." Considering skeletal muscle as an engine for accelerating limbs (inertias) power was calculated for each ninetieth of a second from our results to see how nearly it was constant during the contraction cycle. The figures are given in column 9, table 1. For the graph which relates power and time, see figure 4. It is evident that after the muscle attains its maximal force, power remains approximately constant for a period of about 40 sigma out of a total duration of 100 sigma. Reference to figure 6 will show that force diminishes with increasing velocity during the same time that the power is constant. This relation between tension-loss and increased velocity would necessarily follow from motion under constant power.

Special significance is attached to the results of this series of experiments from the same animal, C481, for the reason that the data have not been smoothed and also because eleven consistent records were averaged together. Examination of the figures in column 9, table 1, shows that the changes in power output during the contraction cycle may be divided into three phases. In the first phase the power expenditure rises from zero to a maximum during a period of 30 to 40 sigma. The phase of constant power approximately 40 sigma long then follows. A third period of about 30 sigma duration occurs when the speed of contraction slackens and force falls to zero. The shortening of the muscle during these three stages is of interest. During the first stage a slight degree of shortening occurs. In cat 481, the maximal force was developed with a shortening of 0.07 cm. The work done during this period is, therefore, relatively small, constituting about 15 per cent of the total. In the second stage, the speed of contraction accelerates and force correspondingly diminishes. About 65 per cent of the total work of the contraction is done during this period. During the third stage of the contraction, the speed of the muscle decreases and force falls to zero. The work done during this phase is about 20 per cent of the total.

In table 3, the variations of power with time are shown for a series of eighteen animals in which the experimental conditions varied considerably as regards the size of the animal, magnitude of the inertia, duration of the response and nature of the stimulus. The velocity curves were all smoothed and the second differences were taken off by measurement. The stimulus was either a tetanus or a twitch. Certain of the tetani were quick releases and in certain others the tension developed after release. Since the duration of the response varied considerably from one set of conditions to another, the figures for power, for each time interval, are expressed as percents of the maximum power for any one contraction, to render them comparable. When these figures are plotted as ordinates on abscissae such that the total contraction is taken as 100, it is evident that a majority of the curves have more or less rounded tops, the flatness of

which indicates the extent to which power is constant during the contraction. These occur with tetani, single twitches and quick releases. The duration of the contraction is obviously a factor in this phenomenon. The longer the duration of the contraction, the more evident is the constancy

TABLE 3

TIME	C474R9 TETANUS I = 290,000	C475R7 TETANUS I = 466,000	C475R7 TETANUS I = 394,000	C477R4 TETANUS I = 495,000	C477R6 TETANUS I = 290,000	C477R7 TETANUS CUT NERVE I = 495,000	C478R1 TETANUS I = 495,000	C484R4 TETANUS QUICK RELEASE I = 495,000	C486Av.7 TETANUS QUICK RELEASE I = 495,000
seconds									
0.011	22.0	11.0	2.9	16.2	37.4	42.7	7.2	3.0	4.8
0.022	66.0	29.9	19.1	45.9	66.0	64.2	26.0	80.6	88.3
0.033	100.0	62.5	56.5	68.3	91.7	81.2	35.6	100.0	100.0
0.044	80.3	100.0	100.0	95.1	100.0	90.9	61.5	79.6	88.0
0.056	44.0	94.1	76.0	96.8	99.5	95.4	82.2	60.9	52.0
0.067	22.0	61.9	51.2	100.0	92.9	100.0	90.8	35.5	16.0
0.078	9.0	15.6	28.7	88.1	81.5	94.9	100.0	18.6	
0.089		6.5	8.2	62.3	54.1	75.7	92.0		
0.100				48.4	28.0	79.7	64.0		
0.111				17.0	14.0	65.7	27.0		
0.122				10.0		29.4			
0.133									

TIME	C488Av.6 TETANUS QUICK RELEASE I = 495,000	C489Av.6 TETANUS QUICK RELEASE I = 495,000	C490Av.11 TETANUS QUICK RELEASE I = 495,000	C495Av.3 TETANUS QUICK RELEASE I = 495,000	C497R1 QUICK RELEASE I = 495,000	C496Av.3 TETANUS I = 495,000	C478R2 TWITCH I = 495,000	C479R2 TWITCH I = 495,000	C479R5 TWITCH I = 495,000
seconds									
0.011	0.7	100.0	22.4	33.5	38.8	88.0	.5.0	21.8	41.6
0.022	22.4	94.0	100.0	100.0	76.0	100.0	19.0	69.5	81.6
0.033	100.0	27.9	54.0	82.0	100.0	88.0	40.9	100.0	100.0
0.044	73.8	10.5	14.9	54.5	96.3	82.0	74.0	92.6	79.8
0.056	15.9	1.4		34.8	75.6	73.3	100.0	76.4	43.4
0.067	13.6			19.8	52.4	69.4	92.6	55.9	20.1
0.078				16.0	36.8	52.0	68.0	17.4	1.5
0.089				6.55	18.4	50.6	45.4	2.2	.05
0.100					9.2	43.7	15.0		
0.111					6.1	30.7			
0 122						18.4			
0.133						3.77			

of the power. This appears especially clearly in experiments 477, 478 and 496.

The results of these experiments lend some weight to the theory that two mechanisms are implicated in the contraction of a muscle. The sudden development of maximal tension within 30 sigma after stimulation with a small degree of shortening may be correlated with an electro-chemi-

cal change characterized by a high speed of evolution (Hill's intensity factor). Coincident with this phase is the rise and fall of the action potential and the initial outburst of heat. With these sudden changes in tension, electrical state and heat, the rigidity reported by Gasser and Hill should also, probably, be grouped. Since these events occur before any considerable change in the length of the muscle takes place, this phase may be designated the isometric phase. With the attainment of maximal tension, however, the second mechanism begins to operate. Correlated with this stage is an increasing shortening of the muscle with an increasing speed. As the speed of shortening increases, force diminishes. The duration of this phase is from 40 to 50 sigma. The action current and heat production are much diminished. Extensibility with a sudden jerk is now considerable. There remains to be mentioned, as a conspicuous feature of this part of the contraction cycle, the approximate constancy of power until the speed of muscle shortening has begun to decrease. By actual measurement in experiment C481, 65 per cent of the total work of the muscle was done under constant power. For this reason, this phase may be called the isoergic phase. For the mechanism operating during the isometric phase, we may postulate an electrochemical process. For the mechanism operating during the isoergic phase we may postulate an elastic process. As regards the elastic factor, our results point to its presence in the approximately linear form of the descending portion of the force-length curve (fig. 5).

It should be borne in mind however that this linear relation may not hold over the whole range of shortening of which the muscle is capable. Furthermore, since force and velocity vary inversely during the isoergic phase some other factor must intervene which modifies the purely elastic process.

SUMMARY

1. A technique is described by means of which the instantaneous tensions and lengths of the gastrocnemius of the decerebrate cat, indirectly stimulated, and contracting against an inertia disc, can be recorded for each eleven-thousandths of a second.

2. The tension-length diagram has been constructed from these data. The work done in a single twitch has also been computed from the kinetic energy of the system. The work done by the muscle during shortening is approximately equal to that of an elastic body shortening through the same distance. $\frac{W}{Tl}$ has been found to be 0.0489.

3. The power of the muscle, calculated for each 0.011 second, is found to be approximately constant for a period of about 40 sigma after the

maximal force has been reached. During this period of constant power, force decreases with increasing velocity.

4. The decrease in force with increasing speed of contraction is interpreted to be a result of the physical law which governs motion under constant power. If power is constant, force must necessarily decrease as velocity increases. Power is shown to be approximately constant during that part of the muscular contraction in which 65 per cent of the work is done. During this period of constant power, force decreases with increasing velocity.

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STUDIES ON NERVE METABOLISM[1]

VII. The Rôle of Lactic Acid

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The discovery of a delayed heat production following activity in frog nerve (Downing, Gerard and Hill, 1926), not unlike that in muscle, at once suggested that a lactic acid cycle might be important in the metabolism associated with activity. The further findings, however, that the delayed heat was not differentially decreased by oxygen lack (Gerard, 1927); that extra production of lactic acid in the presence of glucose did not delay asphyxial block of conduction and that a nerve formed no extra acid when active during oxygen deprivation (Gerard and Meyerhof, 1927); that lactic acid formed in or added to rabbit nerve was not oxidized nor built into carbohydrate (Holmes and Gerard, 1929); that the carbohydrate content was not altered by prolonged activity as compared to an equivalent rest period (Holmes, Gerard and Solomon, 1930); that added lactic acid did not augment the oxygen consumption of frog nerve (Gerard, 1930); these and many similar observations led to the view that the active metabolism of this tissue, in contrast to its resting one, was divorced from a lactic acid mechanism (Gerard, 1932).

The advent of iodoacetic acid, able to block glycolysis, had caused a revision of the picture in muscle and brought into prominence creatin-phosphate as the immediate energy source for contraction. Likewise in nerve, a creatin-phosphate mechanism was established as entering into the active metabolism (Gerard and Tupikow, 1931). Iodoacetate also hastened the asphyxial breakdown of the phospho-creatin, and in sufficient concentration caused a breakdown in the presence of oxygen. These results were in conformity with the view that lactic acid production may serve as an energy source, during anoxia, to maintain the resting nerve machinery in a functional state, even though not directly entering into the actual chemical events associated with activity. A similar interpretation would seem to account for the finding that block of conduction in an anoxic nerve is hastened by addition of iodoacetic acid (Ronzoni, 1931).

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TABLE 1

Frog nerve in 1 cc. Ringer in oxygen. 20°C. Warburg manometers, 4 cc. chambers. Q_{O_2} = cmm. O_2 consumed per hour per gram moist nerve. Alkali in inset. Bracket indicates paired nerves from the same frogs.

	NERVE IN	Q_{O_2} 1–4 hrs.	ADDED	Q_{O_2} 1–3 hrs.	Q_{O_2} 4–6 hrs.	Q_{O_2} 7–12 hrs.	ACTION CURRENT mm.
Nov. 26, 1932	M/22 Na lactate in Ringer	40	M/360 Na iodoacetate	30	28	18	40
	Ringer	39	—	30	33	29	50
	M/22 Na lactate in Ringer	26	—	26	28	28	50
	Ringer	24	M/720 Na iodoacetate	22	21	0	0
		1–3 hrs.		1–3 hrs.	4–6 hrs.	7–10 hrs.	
Dec. 22	M/400 Na iodoacetate in Ringer	18	—	10	7	2	
	M/400 Na iodoacetate + M/22 Na lactate in Ringer	39	—	29	21	12	
	Ringer	44 (2)	M/400 Na iodoacetate	22 (2)	9 (2)	4 (2)	
	M/22 Na lactate in Ringer	48 (2)	M/400 Na iodoacetate	31 (2)	22 (2)	5 (2)	
				1 hr.	2–5 hrs.	6–8 hrs.	
July 10	M/15 Na iodoacetate in Ringer		—	27	0	0	
	M/15 Na iodoacetate + M/30 Na lactate in Ringer		—	35	16	11	

* Duplicate experiments = (2)

Recently Feng (1932) has further reported that nerves blocking rapidly under the action of the halogen acid are considerably protected by the addition of lactate. We have returned to the problem and, while confirming several of the older results, have made new observations supplementing those of Feng. Iodoacetate, even in high dilution (M/700 or

less), depresses nerve respiration; but, especially with low concentrations, the oxygen consumption falls slowly with time for at least six hours. (See Lohmann, 1931; Mawson, 1932, for muscle.) With stronger concentrations the fall is more rapid but even so may show little effect in an hour; and in many cases 0.07 M iodoacetate has less effect than 0.05 M. Nerves with their sheaths split are depressed sooner, but still show a long fall, so that diffusion is not the main basis for the delay. These findings partly account for an erroneous preliminary statement made earlier (Gerard, 1931) that M/15 iodoacetate does not decrease nerve respiration. It should further be stated that the response of individual nerves to this agent is highly variable and the present average results on respiration are to be considered as only roughly quantitative.

Table 1 presents protocols of individual experiments and table 2 a summary of twenty-three pairs. When sodium lactate is added in the

TABLE 2

Summary of experiments on the influence of iodoacetate and lactate on nerve respiration

Na IODOACETATE	Na LACTATE (M/20–M/90)	OXYGEN CONSUMPTION IN PER CENT INITIAL VALUE
M/15	−	0 at 2nd hr.
	+	30 at 7th hr.
M/50	−	0 at 7th hr.
	+	25 at 7th hr.
M/100	−	20 at 7th hr.
	+	35 at 7th hr.
M/300	−	50 at 7th hr.
	+	75 at 7th hr.

absence of the halogen acid it has no effect, but in the presence of iodoacetate it markedly, but not completely, antagonizes the respiratory depression. Thus, even with M/15 iodoacetate, M/30 lactate preserves one-third of the oxygen consumption after seven hours, whereas with no lactate oxygen consumption practically stops in the second hour. Action potentials, slowly abolished by the halogen acid, are also maintained quite well if lactate is present (table 1). After longer times, 10 to 16 hours, respiration and conduction fail even in the presence of lactate.

The respiration of frog nerves at 20°C. is not affected by sodium lactate; at 37°C. it is, if anything, diminished. (Four paired experiments at 37°: Q_{O_2} in Ringer = 120, in lactate = 110.) Dog and rabbit nerves at the higher temperature have given varying results. The average Q_{O_2} values of eight paired experiments on rabbit sciatic were: in Ringer 160;

lactate (M/90-M/20, mainly M/45) 210; glucose (M/100) 235; but the four best, in which known disturbing factors were absent, were: Ringer 140, lactate 140. A progressive decline in respiration of isolated rabbit nerve in Ringer (Sherif and Holmes, 1930) was not usually present, but in two experiments where the fall occurred in Ringer it did not appear when glucose or lactate was present.

The influence of iodoacetate on nerve phosphocreatin was determined by measuring bound creatin (Gerard and Tupikow, 1930). A surprising relation appeared in that the more concentrated halogen acid caused but a small breakdown; the effect increased with dilution to a maximal loss of one-third of the creatin-phosphate in the presence of 0.005 M iodoacetate, and again became less with further dilution (table 3). Addition of lactate, up to M/9, did not alter the creatin-phosphate of nerves in Ringer, nor did it in any way protect against the breakdown induced by iodoacetate (table 3). Fortunately, the experiment was tried further, using nerves with the sheath split, under which conditions a definite protective action of the lactate is manifest—the phosphocreatin breakdown that occurs with the halogen acid alone being largely prevented by the presence of lactate (table 3). Two similar experiments performed in hydrogen failed to demonstrate any influence of lactate on the breakdown induced by anoxia and iodoacetate. It has been shown that tetanization leads to a breakdown of 5 mgm. per cent of creatin phosphate (Gerard and Tupikow, 1931). Additional experiments in the presence of the iodoacetate now yield the same or smaller figures (table 3), so that the halogen acid does not increase the phosphocreatin breakdown of activity.

The present results, as well as those of Feng, strongly suggest that an iodoacetate poisoned nerve, and presumably a normal one even more, is able to oxidize lactic acid. It *may* then be possible that glucose passes through a lactic acid stage in its oxidation. That it *does* so, however, is not a necessary consequence; nor is there any positive evidence that lactate enters into the chemical mechanism of conduction. It would be consistent with the new as well as the earlier findings that, although lactate may be burned when other oxidations are interfered with by the halogen acid, it is ordinarily not attacked even when present because the oxidative machinery is occupied by more readily available substrates. Thunberg's finding (1923) that nerve brei, washed free of substrates, reduces methylene blue after lactate addition is suggestive of this view.

The point has been made elsewhere (Gerard, 1932) that experimental conditions may influence nerve conduction not only by a direct action on the metabolism of activity but also indirectly by affecting the resting metabolism and so the "set" of the activable system. The present results may be consistently interpreted in terms of the second possibility. The concentrations of iodoacetate used by Feng and ourselves markedly in-

terfere with the resting oxidations of nerve, which are known to be in large part of carbohydrate. For muscle and brain it is known (Meyerhof and Boyland, 1931; Krebs, 1931) that the halogen acid blocks carbohydrate oxidation while permitting that of lactic acid; and for nerve, likewise, it

TABLE 3

TREATMENT	PHOSPHOCREATINE IN TREATED NERVE AS PER CENT OF CONTROL (RINGER)	NUMBER OF EXPERIMENTS
	per cent	
2.5 hours in oxygenated solution of Na iodoacetate in Ringer		
0.073 M	90	5
0.020 M	75	2
0.005 M	70	9
0.002 M	75	2
0.0005 M	80	2
Same, but 0.11 M Na lactate in Ringer	100	3

TREATMENT	BOUND CREATIN			NUMBER OF EXPERIMENTS
	Iodoacetate	Iodoacetate + lactate	Change	
	mgm. per cent	*mgm. per cent*	*mgm. per cent*	
2.5 hours in oxygenated Ringer + 0.005 M Na iodoacetate or same + Na lactate (0.01–0.05 M)	42	42	0	10
Same; nerve sheath split; iodoacetate added 10 minutes after start	39	48	+9	5

TREATMENT	REST	STIMULATED	CHANGE	NUMBER OF EXPERIMENTS
2 hours in oxygenated Ringer				
No iodoacetate			−5	12*
No iodoacetate on resting nerve; 0.005 M on active	43	39	−4	4
0.01–0.002 M iodoacetate on both nerves	44	39.5	−4.5	4

* Previous results.

appears that added lactate is oxidized by the iodoacetate poisoned nerve. The halogen acid, then, would act at least in part like anoxia or cyanide to suppress oxidations in the resting nerve; the lack of oxidative energy in turn results in loss of phospho-creatin, change in physical state of membranes, etc., and so in inactivability or block of conduction. Lactate

addition, by re-initiating the oxidation sequence, tends to offset the poisoning effect. In harmony with such an interpretation, as alternative to the view that lactic acid formation and removal normally accompany nerve conduction, are the facts: that the creatin-phosphate loss on continued tetanization is no greater in poisoned nerves than in untreated, though the halogen acid does itself cause a loss in the resting nerve; that lactate addition does not influence the anoxic failure of the treated nerve though it does improve the recovery in oxygen (Feng, 1932); that the course of heat production of activity is not altered by iodoacetate (Feng, 1932); that the resting oxidations in nerve are largely of carbohydrate, those of activity apparently not; and many other observations summarized above. It is not clear why in the unsplit nerve added lactate counteracts the action of iodoacetate on respiration and conduction but not on creatin-phosphate breakdown, nor why the higher concentrations of the halogen acid have relatively less effect on the creatin-phosphate than on oxygen consumption.

Both Gerard (1932) and Feng (1932) estimate a maximal continued heat production of activity by frog nerve at 0.1 cal. per gram per hour, which would correspond to the oxidation of 8 to 9 mgm. per cent of glucose in three hours. Though it has been suggested that such a quantity might have been missed in the experiments performed to determine any carbohydrate loss due to activity and which gave negative results, this seems most improbable on examining the actual results obtained.[2] Further, similar absence of sugar usage was found in frog nerve over a period three times as long and in rabbit nerve under conditions that called for a loss of five to ten times this quantity of glucose. It still seems preferable, therefore, to consider the chemical events of nerve conduction as independent of a step involving lactic acid; but the new possibilities opened up, if lactic acid can be oxidized by nerve, necessitate a further experimental analysis of the question.

We are indebted to Miss N. Tupikow for assistance in the creatin determinations.

[2] Table 3 (Holmes, E. G., R. W. Gerard and E. I. Solomon: 1930, This Journal, xciii, 342) includes sugar determinations on 20 pairs of nerves, stimulated and at rest. Excluding one aberrant pair, the difference between the resting and stimulated means is −0.63 mgm. per cent, and the standard error of the mean difference is 1.26. Or, in terms of Fischer's test for the homogeneity of two populations (*Statistical Methods for Research Workers*. London, Oliver and Boyd. 1925), $t = 0.50$; and the probability of the two sets of data, rest and stimulated, belonging to the same series is over 0.6. Of the nineteen pairs of observations, in only three cases were the differences between rest and stimulated nerves as much as 9 mgm. per cent, plus or minus.

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EFFECTS OF ADRENAL MEDULLA AND THYROID ON THE RESPIRATORY METABOLISM OF PANCREATIC DIABETES

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In hyperthyroidism and after stimulation of the adrenal medulla, the respiratory metabolism is increased and the blood sugar is raised. Pancreatic diabetes shows similar changes, a fact which suggests that either the thyroid or the adrenal medulla or both may be more active following destruction of the islet cells.

Adrenal glands and diabetes. Frouin (1908) showed that removal of three-quarters of the adrenal glands diminishes the severity of diabetes following pancreatectomy. Mayer (1908) found that complete removal of the suprarenal glands produced similar results though the animals survived for only a short period of time. Mackenzie (1917) declared that adrenalectomy in diabetic dogs causes a disappearance of the hyperglycemia. This result was confirmed by Turcatti (1929), but Hédon (1921) stated that the hyperglycemia is affected little or not at all if the adrenalectomy occurs after the pancreatectomy.

All of the above results were complicated by the removal of the cortex as well as the medulla, and since the medulla is the part of the adrenal gland which will raise blood sugar and increase metabolism, we may now consider experiments in which the cortex was not removed. Houssay and Lewis (1921) and Turcatti (1929) noted that pancreatic diabetes continues after adrenal demedullation. Also, Stewart and Rogoff (1923) have shown that in depancreatized dogs with adrenal inactivation, the blood sugar was as high as 0.29 per cent. They explained that the blood sugar in diabetic animals fails to rise after adrenalectomy because of the moribund condition. On the other hand, Boeggild (1923) believed that destruction of the adrenal medulla by cauterization can diminish the diabetic hyperglycemia. Ciminata (1928) declared that he can cure pancreatic diabetes by denervation of the suprarenal glands. After such a procedure, he says that the blood sugar returns to normal, the glucose tolerance increases and the body weight becomes greater.

Thyroid and diabetes. In 1896, Minkowski noticed that thyroid feeding in dogs produced glycosuria. He then experimented with removing the thyroid glands from dogs and later depancreatizing them, but this did not

diminish the symptoms of diabetes. The result is in contrast to the work of Lorand (1904) who found in diabetic dogs an increase in the colloid substance of the thyroid vesicles. Eppinger, Falta and Rudinger (1908), after removing the pancreas and thyroid, found protein metabolism unchanged instead of being increased three or four fold as in simple diabetes. Lusk (1913) and Dann, Chambers and Lusk (1931) obtained similar results in phlorhizin diabetes. Thyroidectomy diminished or prevented the increase of protein metabolism and of total heat production occurring after phlorhizin in unoperated animals. Pancreatectomy after thyroidectomy raised the respiratory metabolism 20 per cent in one animal. Friedman and Gottesman (1922) reported that glycosuria in depancreatized dogs was checked after complete ligation of the thyroid arteries. Partial occlusion, however, apparently intensified the diabetes produced by pancreatectomy. Allen (1922) also showed that thyroid deficiency may reduce or abolish the glycosuria or hyperglycemia of diabetes. He believed, however, that this did not indicate that the thyroid contributes to the causation of diabetes. Wilder (1926) stated that "the phenomena exhibited by patients with diabetes combined with states of hyperthyroidism or hypothyroidism may be related to the general metabolic rate and thus may be explained without recourse to speculation as to the specific interdependence of thyroid and pancreas." Wolfson (1927) showed that "thyroidectomy in diabetic animals without insulin treatment is ineffective in lowering blood-sugar levels. Urinary sugar excretion diminished but the animals never became aglycosuric." Shpiner (1927) pointed out that sodium nucleinate produced the same effect on blood sugar and metabolism as thyroxin, and he believed, therefore, that the thyroid influenced carbohydrate metabolism only through its effect on basal metabolism. Yriart (1930) from evidence on urine and blood sugars concluded that thyroidectomy did not modify the development of pancreatic diabetes.

Method. The measurements of metabolism were made in exactly the same way as described in our previous paper (1932). The pancreas was removed by tearing the tail free from its mesentery and rubbing the head away from the blood vessels going to the duodenum, being careful to tie the pancreatic duct as soon as it was located. We made certain that the small piece of pancreatic tissue surrounding the portal vein was removed and that no nodules were left attached to the small intestine. Usually the pancreas came out as a single strip. The "inactivation of the adrenal glands" consisted of removal of the right adrenal, the cutting of the major and minor splanchnic nerves on the left, and the removal of the left sympathetic chain from the diaphragm to below the level of the kidney.

At first we found it very difficult to obtain long survival in such animals because of their extreme sensitivity to insulin (Cannon, McIver and Bliss, 1924). Our most successful procedure consisted of giving the cats 1 unit

of insulin in the morning of the second day after the operation, and supplying fresh food at this time. Then 0.5 unit of insulin was injected at noon and at night. Later the animals received 2 or 3 units in the morning and two injections of 0.5 unit each later in the day. The cats gradually lost weight but the metabolic determinations were similar to ordinary diabetic animals. The loss in weight seems to be of little importance since the diabetic cats previously reported (Ring and Hampel, 1932) lost occasionally 40 per cent of their weight without any change in metabolism during the loss.

For the other part of our studies, we determined the normal metabolism before thyroidectomy and then one week or more following this operation when the new level of heat production had been established. The final metabolic measurements were made after recovery from the pancreatectomy.

Results of adrenal inactivation. Since the results here reported show no effect upon the metabolism of diabetes with inactivation of the adrenal medulla, it is important to know that the operation has been successful. About this we are certain, first because of the care we used while inactivating the adrenal medulla, and second because of the marked sensitivity to insulin which the cats displayed. Three of our animals on the day after pancreatectomy received 0.5 to 0.7 unit of insulin per kgm. and developed a hypoglycemia so quickly that they died before treatment could be effected. A fourth, cat 472, was saved by giving an intravenous injection of glucose. Ordinary diabetic animals do not develop any hypoglycemic symptoms upon receiving 1.5 unit of insulin per kgm.

Four cats upon which normal metabolisms were determined prior to the operations gave results between 28.3 and 32.3 calories per square meter per hour. After recovery from adrenal inactivation and pancreatectomy, the animals showed essentially unchanged metabolisms while receiving insulin, namely, 28.3, 30.2, 32.3 and 33.3 calories. Without insulin, the metabolisms increased to 39.0, 41.2, 42.5 and 43.3 calories. The average of these results and those from other animals on which the data are less complete was 41.7 $\pm$ 0.6. The respiratory quotients averaged 0.726 $\pm$ 0.006. In uncomplicated diabetes, our results were 41.7 $\pm$ 0.4 calories and 0.70 $\pm$ 0.004 for the respiratory quotient.

There is no doubt in our minds that the diabetes in these cats was just as severe as in animals without the adrenal medulla inactivated. For example, cat 472, mentioned above, showed on the fourth day after being deprived of insulin a marked glycosuria and an acidosis, developed an ataxic gait, vomited, and failed to eat. We felt that the animal would die if not given insulin on that day.

Results of thyroidectomy. We were surprised to find that the removal of the lateral lobes of the thyroid in cat 480 produced only an insignificant

change in metabolism. Cat 476 showed a decrease of 12 per cent and cat 466 a fall of 27 per cent below the normal. These differences in response to thyroidectomy are probably due to the amount of accessory thyroid tissue remaining. Marine (1922) states that "in cats and dogs accessory masses may be demonstrated in upwards of 90 per cent." According to Otto (see Hirsch, 1932), the isthmus is found in young cats but degenerates in old age.

In all five animals, pancreatectomy produced a marked increase in the level of metabolism found after the lateral lobes of the thyroid had been removed. This rise was present not only in the cats showing slightly reduced metabolisms but also in those whose heat production dropped so low that it seemed unlikely any thyroid remnants could have remained. For instance, cat 466 showed a 32 per cent increase, and cat 482 a 122 per cent increase. The rise in cat 480 was 31 per cent and in cat 476 it was 31 per cent. These results compare favorably with the average increase of 42 per cent found in a large series of cats with uncomplicated diabetes. Certainly we are assured that the thyroid does not play a major part in raising the metabolism after pancreatectomy.

CONCLUSIONS

Inactivation of the adrenal medulla failed to bring about a lowering of the high metabolic rate of diabetes. The average basal rate for normal cats was 29.3 calories per square meter per hour, for uncomplicated diabetes it was 41.7 calories and after inactivation of the adrenal medulla it was still 41.7 calories.

Thyroidectomy likewise does not prevent the usual increase in metabolism following pancreatectomy.

This work was started at the suggestion of Dr. W. B. Cannon and he has constantly helped with suggestions.

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THE OXYGEN CONSUMPTION OF STIMULATED NERVE

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With the recent advances in the field of nerve physiology brought about by modern methods for investigating electrical, thermal, and metabolic phenomena has come a realization of the desirability of still further decreasing the number of unknown variables in any given experiment by combining techniques. Simultaneous measurement of the electrical, thermal and metabolic changes in a single nerve would be ideal. This seems impossible of accomplishment in spite of the great improvements made by Hill and his co-workers in neurothermic methods. However, it is possible to effect a combination of the electrical and metabolic methods. Fenn (1927) reported experiments in which the gaseous interchange of a single green frog sciatic and its action potentials as measured galvanometrically were recorded simultaneously. Recently (Schmitt, 1932) we reported experiments in which simultaneous readings were made of the electric response, recorded oscillographically, and of the gaseous metabolism measured by an improved volumetric method. The numerous difficulties of this technique have now been sufficiently overcome to warrant publication of the details of the method. In the present paper we also present experimental evidence bearing on the question raised by Winterstein as to whether the increased oxygen consumption of stimulated nerve is an artifact arising from the stimulus or is a physiological effect attendant on the propagation of the impulse down the nerve. It would seem that this question could be answered only by a quantitative method such as will be described in detail below.

1. *Respirometer.*[1] Following the lead of Fenn we have turned to the volumetric method of measuring oxygen consumption rather than the manometric method because of its greater sensitivity and because the sensitivity does not depend upon the volume of the vessel.

This latter point is important for it is often necessary to design the electrodes in a way such as to require relatively large vessels. This would

[1] I take pleasure in acknowledging my indebtedness to Mr. W. J. D. Walker, of Washington University, for his valuable services in constructing the numerous varieties of vessels which were tested in the course of our search for the most desirable type.

be impossible with the manometric method for the loss in sensitivity occasioned by the large vessel size (ca. 5 cc.) would rule it out.

Figure 1 shows the type of respirometer used in most of the experiments. A bridge stopcock is interposed between the two vessels as a shunt for the capillary. This stopcock serves to protect the droplet in the capillary from sudden changes in pressure in either of the vessels and also facilitates the passage of gas through the vessels. In later designs of vessels we have even omitted the two side stopcocks; in this case the bridge stopcock can be seated only after the two electrode stoppers have been inserted. The elimination of the two side stopcocks simplifies the apparatus and tends to prevent a certain amount of apparatus drift which seems to arise from the turning of stopcocks. This type of respirometer has the drawback, of course, that gas cannot be perfused through the vessels.

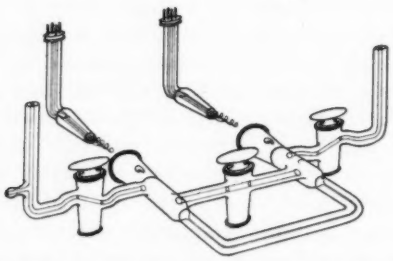

Fig. 1. Respirometer with electrodes. Spur on left hand tube is for purpose of passing gas saturated with Ringer solution and equilibrated for temperature through the vessels via the bridge stopcock.

A relatively large capillary bore was adopted (0.4–0.5 mm.) in order to avoid sticking of the droplet and other irregularities in its movement resulting from surface phenomena. Then, in order to obtain high sensitivity, refinements were necessary in the method of reading the position of the droplet. A travelling microscope was constructed as shown in figure 2. With a 16 mm. objective and a 6× ocular fitted with a cross hair, the meniscus of the droplet can be brought very nicely into focus. Illumination is furnished by a minute electric lamp enclosed in a glass bulb and suspended from the moving block carrying the microscope. The glass bulb is painted black except for a narrow cross slit to avoid differential heating effects due to absorption of light by the vessels. The microscope block is screwed to a lug which engages an acme screw thread. This screw thread is very accurately machined and has a pitch of one-tenth inch. The rotation of the screw thread and hence the excursion

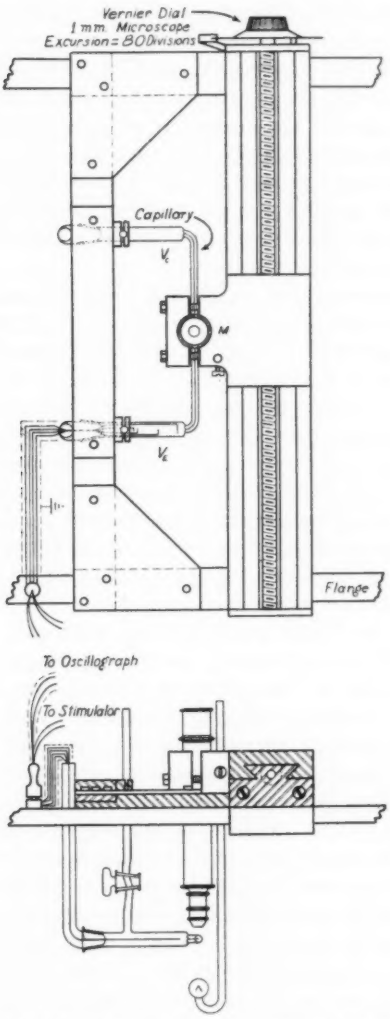

Fig. 2. Top and side views of respirometer suspended from steel holder with reading microscope in place. The surface labelled *Flange* represents the flanged upper side of the cast iron thermostat. M, reading microscope; V_C and V_E control and experimental vessels respectively. The respirometer shown in this figure is of earlier design than that shown in figure 1 and lacks the desirable bridge cock feature. The steel support is so made that any respirometer may be adapted to it. When high amplification of the action potential is required it is best to attach the radio plug leading to the electrode wire directly at the end of the glass electrode stopper as shown in figure 1 rather than through the intermediate plug on the steel support; this procedure greatly reduces AC pickup and is essential for work with late waves of low potential.

of the microscope is read off accurately by the vernier dial[2] at the end of the screw. This dial can be read to 0.1 division and is graduated in 200 divisions per revolution. Hence the position of the microscope can be determined accurately to about the nearest micron. If the capillary bore is 0.4 mm., assuming that the vessels are of equal volume, the sensitivity of the apparatus is approximately 5×10^{-7} cc.

Filter paper strips soaked in an N/8 solution of NaOH are placed on the walls of the vessels so as to cover from 60 to 80 per cent of the exposed surface of the vessels. This serves to absorb CO_2 and also to maintain the proper vapor pressure in the vessels.

Although kerosene was used as the index droplet in most of the experiments, numerous other liquids were also tried. Surface tension, viscosity and vapor pressure data were obtained for over fifty organic compounds with a view to obtaining a fluid better suited to this purpose than kerosene. While viscosity and surface tension are the most important factors which determine the flow of the droplet through the capillary, nevertheless, if the vapor pressure is appreciable, the droplet will drift slowly through the capillary of itself, due presumably to the unequal vaporization of the fluid from the ends of the droplet. This factor immediately reduces the number of liquids which might do well in the capillary. We have found that dimethyl aniline, butyric acid, valeric acid, and perhaps three or four other substances serve well. The chief difficulty with kerosene seems to be the presence of gums and highly polymerized compounds which exert an appreciable lateral tug on the droplet owing to preferential deposition on the surface of the capillary. To avoid this the kerosene must be washed thoroughly with concentrated sulfuric acid for days, neutralized, dehydrated and distilled. We have had very satisfactory results from the use of such especially prepared kerosene.

In order to decrease the period necessary for equilibration, immediately after being placed in the thermostat the respirometer is perfused with gas saturated with water vapor at the temperature desired. Oxygen, or whatever gas is to be used, is passed through a special bottle submerged in the thermostat, by means of which the gas is equilibrated with Ringer solution contained in the bottle. Thence, after having passed through about twelve feet of small-bored copper tubing also submerged in the thermostat, the gas is led into the respirometer through the small spur tube just beyond the left side stopcock, the bridge cock being open. After allowing fifteen minutes for this equilibration both side stopcocks are closed. Then after a further period of 15 to 30 minutes the bridge stopcock is closed and readings of respiration commenced.

Standard interchangeable ground joints are used throughout; this

[2] Precision Velvet Vernier Dial, Type N, obtained from National Co. Inc. of Malden, Mass.

makes it possible to use electrode stoppers, plain stoppers, or specially shaped stoppers interchangeably on any of our vessels. All glassware is of pyrex. For the electrodes, tungsten wires are sealed into the pyrex joint and extended the required distance into the vessel and perhaps 2 cm. back into the ground joint. Thin copper wires communicated to the four-pronged radio plug above the surface of the water bath. This plug served as a convenient method of connecting the electrodes to the oscillograph and stimulator leads. The actual electrodes on which the nerve is laid consist of small platinum rings spotwelded to the tungsten wires. A film of Ringer solution placed in the rings decreases the strength of shock necessary to excite the nerve, and thus decreases the heating effort of the shock. Furthermore the electric paths of the escaping currents are greatly restricted by the use of these symmetrically shaped electrodes and the shock artifact is reduced to a minimum.

2. *Thermostat.* When the extremely sensitive volumeter described above was put into use it was found that the kerosene droplet moved through the capillary unevenly in response to the nerve respiration. This irregularity was not very noticeable when the readings were made only every 10 or 15 minutes, but if readings were made every 2 or 3 minutes, this "pumping" movement of the droplet became a limiting factor of accuracy. It was found that the cause of this irregularity of movement was to be assigned to rapid local fluctuations of temperature in the vicinity of the vessels. To eliminate this difficulty a new type of thermostat was evolved which was aperiodic and in which the occurrence of temperature pockets was reduced to a minimum (Schmitt and Schmitt, 1932a). When the heating and cooling units are properly adjusted and the difference in temperature between the prethermostat and main thermostat is correct for the desired temperature the apparatus automatically thermostats without further attention indefinitely to better than 0.001°C. Temperature pockets are almost completely eliminated as is evidenced by the fact that the droplet moves evenly through the capillary with no sign of "pumping" movements and 3 minute readings are practically as accurate as 15 minute readings. This refinement makes it possible to obtain numerous readings during relatively short intervals and thus to follow with more surety respiratory processes which may be of short duration.

3. *Stimulator.* At the outset of the work we attempted to use induction shocks and condensor discharges as a means of stimulation. While this method was fairly satisfactory for low rates of stimulation it was decidedly unsatisfactory for rapid stimulation, producing great irregularities both as to the strength of shock and as to the spacing of the shocks in time. At subthreshold or at just-threshold intensities such mechanical systems give particularly unsatisafctory results, for mechanical keys do not close or open exactly the same each time. To obviate these difficulties, an

entirely non-mechanical vacuum tube stimulator was constructed (Schmitt and Schmitt, 1932b). This stimulator has proven extremely satisfactory in every respect, particularly because of its flexibility and the ease with which a change from rapid to slow stimulation may be made, and because of the extreme accuracy with which the intensity of the shocks can be controlled. Since the stimulator gives "all or nothing" shocks it is possible to calibrate the intensity of stimulation quantitatively—in volts if so desired—even at rates of stimulation as high as three or four hundred shocks per second. This is of utmost importance in such experiments as those described below in which the absolute stimulation energy must be known.

The secondary of the coil is designed to permit easy interchange with coils of different constants. This is important if one is attempting to determine the metabolic consequences of stimulation of the smaller *B* and *C* fibers. These fibers fatigue very rapidly when stimulated with fast shocks but can be worked with very well if the shock duration is greatly increased by inserting a properly designed secondary in the stimulating coil.

4. *Operation.* In performing an experiment the thermostat is first brought to temperature equilibrium. To maintain temperature at 20°C. in the main thermostat, we have found it best to regulate the prethermostat at a temperature about 0.4° lower, e.g., at 19.6°C. Having brought the temperature of the prethermostat and of the main thermostat to 19.6° and 20° respectively, the resistance R_1 to control the maximum variable heating (see Schmitt and Schmitt, 1932b) and resistance R_2 to control the constant heating are adjusted properly. The system is now left to itself to equilibrate. When the thyratrons begin flickering constant temperature will have been achieved; meanwhile the nerve is dissected, weighed and placed in the vessel. In experiments on the after-potential the nerve is burned between the two lead-off electrodes to render it monophasic; this operation does not seem to cause any great irregularity in the respiration.

The glass tube of the electrode stopper through which the wires are brought out to the radio plug connector (see fig. 1) is filled with transil oil to prevent sudden changes in room temperature from affecting the experimental vessel. If the temperature of the thermostat is more than a few degrees above or below room temperature it is necessary to equilibrate this oil with the temperature of the bath before placing it in the electrode stopper. Failure to observe this precaution results in a negative drift in the movement of the droplet since final temperature equilibration may require a period of many minutes. One must also very carefully avoid the contamination of this oil with traces of water, for this produces distortion of the action potential shape and also acts as a source of A. C. pickup.

Having placed alkali-soaked filter paper on the walls of the vessels the nerve is placed in the electrode rings and the greased ground joints shoved home. Much depends on the proper greasing and insertion of the ground joints. It seems that the best method is to smear a small ring of lanolin or similar substance near the end of the ground joint and insert it without pushing the joint together too hard. Some of the apparatus drift to be discussed presently is due to the slow differential squeezing of grease into the ground joint by one or the other of the joints.

Carefully purified kerosene seems to be as desirable as any fluid for use in the capillary. One must, however, allow the droplet to move very slowly back and forth in the capillary just before closing the bridge cock, else the movements of the droplet may show irregularities and drift.

In the early experiments it was found that the rate of decrease of volume in the experimental vessel after starting at a high value, subsided after an hour or so to a relatively constant value. However, even thereafter the rate continued to fall off usually from 2 to 10 per cent per hour, and only after some hours if at all did the rate arrive at a truly constant value. Most of this decrease, especially in the early minutes of the experiments we attribute to apparatus drift, for exactly similar phenomena can be demonstrated to occur if the apparatus is similarly prepared but the nerve omitted from the vessel.

Realizing the importance of eliminating this drift we have investigated the various factors which might contribute to the phenomenon. Space does not permit of a detailed presentation of the numerous experiments performed to evaluate the importance of the various factors involved, but a few of the conclusions might be mentioned for the benefit of those interested in the technique.

If one places the respirometer in the thermostat without the nerve and without the wet filter paper on the walls of the vessels, one invariably observes an appreciable movement of the droplet which may persist at a high but irregular rate for hours. An important factor in this case is the differential condensation of water vapor on the walls of the glass vessels; it is especially prominent if the temperature of the thermostat is considerably lower than room temperature. This is eliminated if filter paper strips wet with water or with alkali are placed on the walls of the vessels, as is the case when respiration is being measured. Drift due to temperature equilibration of the transil oil in the electrode stopper and to differential squeezing of grease into the ground joints has already been mentioned. If kerosene is used there is little danger that a differential evaporation from the two sides of the droplet may be sufficiently rapid to cause small irregularities in the movement of the droplet, though the presence of gums and residues in the kerosene may cause small surface effects.

In the earlier designs of electrodes used by us, the wire was of fairly large diameter. When such electrode stoppers were used, a definite correlation between room temperature and certain wave like variations in the rate movement of the droplet could be demonstrated. To avoid this, very light wires were used to connect the electrodes in the vessel to the plug connection above the water surface.

Occasionally, in the past two years, we have observed certain transient disturbances in the respiration curve which seemed to be caused in some remote way by electrical effects. Merely turning on the amplifier or thyratron filaments, or by passing current intermittently through an induction coil not connected to the nerve caused the droplet to show irregular movement. A single spark discharge of a condenser in another room may produce irregularities in the movement of the droplet which may last for many minutes. Since it does not seem to be a question of a mere physical effect on the apparatus, the important possibility arises that the effect is physiological and as such may be a complicating factor in all experimental work with living tissues. A full report of the investigations on this subject is reserved for separate publication.

THE OXYGEN CONSUMPTION OF STIMULATED NERVE.[3] From the above description of the apparatus and method, it will be apparent that we are now possessed of a sensitive tool for the investigation of the metabolic significance of the electric phenomena in nerve which modern oscillographic technique has, in the last decade, been revealing. The first problem to which we have turned our attention is that which has arisen out of the controversy initiated by Winterstein regarding the extra oxygen consumption of activity of peripheral nerve. Winterstein believes that while it is true that nerve stimulated to activity over electrodes inside the vessel shows marked increase in oxygen consumption, stimulation outside the vessel produces little or no increase in respiration. He distinguishes then between artificial stimulation (*Reizung*) and physiological stimulation (*Erregung*) and holds that under physiological conditions, the passage of the impulse in nerve is not accompanied by an increased gaseous metabolism although it is accompanied by increase in sugar utilization and by other chemical reactions. For summaries of the two opposing viewpoints the reader is referred to the reviews by Winterstein (1929, 1932) and Gerard (1932).

Winterstein was doubtless greatly influenced by the apparent relation between the strength of stimulation and the excess oxygen consumption (Winterstein and Hirschberg, 1927; Ledebur, 1927). And this point is

[3] An abstract of this material was read before the XIVth International Physiological Congress in Rome. The author avails himself of this opportunity to thank the American Physiological Society for the International Physiological Congress Fund Fellowship which enabled him to attend the Congress.

fairly crucial to the argument. If it can be demonstrated that the excess oxygen consumption runs parallel with the intensity of stimulation from all grades of subthreshold strengths through threshold and maximal intensities, the contention of Winterstein would receive strong support. If, on the other hand, the excess oxygen consumption not only fails to show such a parallelism but on the contrary shows a parallelism between the action potential and the excess oxygen consumption, the Winterstein view becomes inadmissible. We refer advisedly only to shock intensities up to just-maximal values, for everyone will admit that there may be rough parallelism between shock strength and oxygen consumption when the shock is strong enough to produce unphysiologic and irreversible chemical changes in the nerve.

Data such as these have not been available in the past because of a lack of quantitative control of shock intensity and from lack of precise measurement of action potentials. The experiments now to be described indicate conclusively that the parallelism exists between the excess oxygen consumption and the action potential rather than between it and the intensity of stimulation.

EXPERIMENTAL. The relationship between the oxygen consumption, shock strength and action potential has been investigated along three distinct lines: 1, by reversal of direction of stimulation holding shock strength constant; 2, by starting with subthreshold strengths of stimulation and increasing slowly the strength of stimulation until a maximal value is reached; 3, by maintaining the intensity of stimulation constant but varying the rate. These experiments will now be described more fully.

1. *Direction of stimulation reversed, intensity held constant.* We observed in numerous experiments that mere reversal of the direction of stimulation without change in applied voltage caused the action potential to be greatly decreased or to disappear entirely. This effect is attributable to dropping out of fibers caused by an increase in threshold when the cathode lies on terminal tissue distal to the lead-off electrodes, and to some extent to anodal block.

Since intensity of stimulation is constant regardless of the direction of stimulation this would seem to afford a method of testing the validity of Winterstein's hypothesis. Data from a typical experiment are plotted in figure 3. These experiments clearly show that excess oxygen consumption follows the curve of action potential rather than that of stimulation intensity. The possible objection that the current passing through the nerve in one direction may not be the same as that passing through in the reverse direction is ruled out by a consideration of the principle of the thyratron stimulation. A more cogent objection would be one concerning the possible effect on respiration of the electrotonic currents which flow under the condition of anodal and cathodal polarization. If a prepon-

derating cathodal escape current stimulates respiration more than a similar anodal escape current, it would be possible to explain such experiments as are illustrated in figure 3 by this function of the stimulating current rather than by insisting on the parallelism between action potential and respiration. The answer to this argument is to be found in the response of the nerve at subthreshold intensities when the direction of stimulation is reversed. If asymmetry of polarization is to explain the

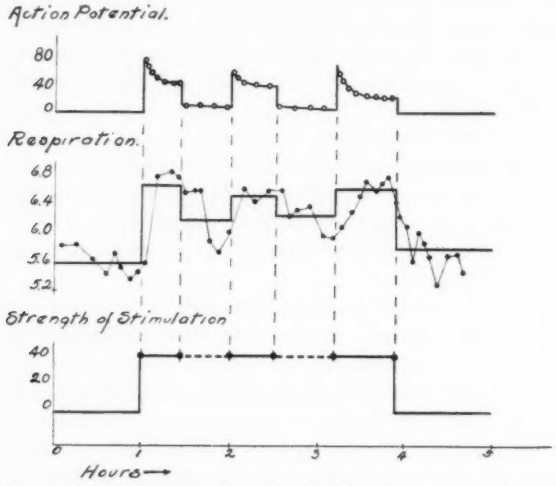

Fig. 3. Effect of reversing direction of stimulation on the excess oxygen consumption. March 28, 1932. Ordinates: action potential in mm. on Braun tube; respiration in divisions per minute (1 division = 0.0125 mm.), strength of stimulation in units; abscissae, time in hours. Dots on respiration curve represent actual rate readings, heavy lines represent average rate for the entire period. Solid lines on stimulation curve indicate that the cathode lies proximal to the lead-off electrodes and yields high action potentials, broken lines indicate the opposite polarization yielding low action potentials. Weight of nerve, 21 mgm.; bore of capillary, 0.507 mm.; temperature 20.5°C. Direction of stimulation was reversed by throwing switch *H* (see Schmitt and Schmitt, 1932b). Rate of stimulation = 180 shocks per second.

data, then similar, although perhaps less striking, effects must be apparent upon reversing the direction of stimulation at just subthreshold intensities. No such effects have been observed experimentally. In some twelve satisfactory experiments in which such subthreshold reversal was tried, there was no consistent difference in the effect on respiration whether the cathode or the anode was proximal to the lead-off electrodes. In order to avoid residual polarization, the stimulator has been so designed that the shock is actually diphasic, the quantity of electricity in the two phases

being roughly equal. Hence we must conclude that the differences observed at submaximal strengths were due to a dropping out of fibers and as such supports the view that the excess metabolism depends qualitatively at least upon the production of action potenitals.

2. *Gradual increase in shock strength.* The quantitative nature of the present method makes possible a critical test of the experiments of Winter-

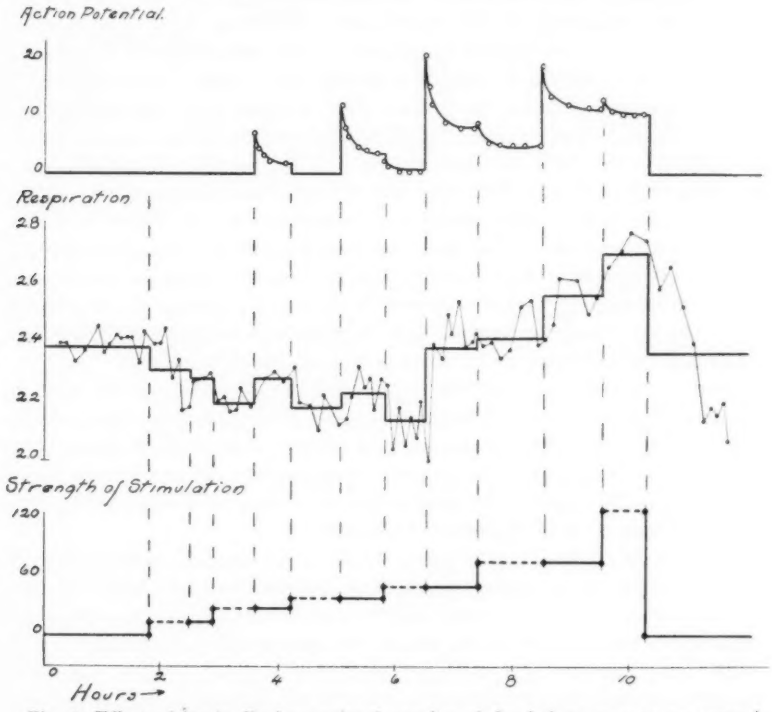

Fig. 4. Effect of gradually increasing intensity of shock from zero to supermaximal value. April 13, 1932. Coördinates as before. Weight of nerve, 20.9 mgm.; temperature, 16°C.; bore of capillary, 0.507 mm. Polarization of stimulation indicated by solid and broken lines as before. Note that increases in respiration occur only when action potentials are produced.

stein and Hirschberg (1927) which indicated that the excess oxygen consumption varies roughly as the shock strength. In the present series of experiments the shock strength was increased in gradual steps, starting with a value about one-third threshold and increasing to just-maximal, and in a few experiments to super-maximal values (see fig. 4). If the oxygen consumption depends primarily on quantity of shock one would

expect it to increase gradually as the shock energy is increased. Instead, one sees that increases of oxygen consumption are noted only when action potentials are produced and in an amount roughly proportional to the action potential, rather than to the intensity of stimulation. It should be noted in figure 4 that subthreshold stimulation produces a *decrease* of respiration. Now it is certain that a portion of this effect is contributed to by apparatus drift for if drift is present at all it is particularly noticeable in the beginning of the experiment. However, after allowing for drift it was found in twelve experiments that subthreshold stimulation may produce a *decrease* in oxygen consumption of from 1 to 5 per cent of the average resting rate. In several other equally good experiments no such inhibiting effect of subthreshold stimulation could be demonstrated. It is possible that this decrease may have some bearing on the discovery of Erlanger and Blair (1930) that subthreshold stimulation of the appropriate characteristics may produce a depression in irritability in nerve. How this local effect at the electrodes could produce a change in the respiration of the whole nerve, however, is not clear. Since we are dealing here with extremely small effects near the limits of accuracy of the method, and since the effects are not always demonstrable we propose to withhold opinion for the present as to the meaning of the phenomenon.

In any case there is no *increase* in metabolism with subthreshold stimulation as there should be according to Winterstein's hypothesis. Incidentally figure 4 also demonstrates the striking effects of reversal of direction of stimulation on the excess oxygen consumption. These experiments clearly show that there is no increase in respiration until the shock is just strong enough to produce action potentials.

3. *Intensity of shock held constant but frequency varied.* Another way of demonstrating the independence between metabolism and energy of stimulation is the following. In the beginning of the experiment a preliminary exploratory search is made to determine the strength of stimulation required to maintain the action potential maximal for a given frequency (usually around 100 per second). Then after allowing a period for rest, the nerve is stimulated with this predetermined intensity but with a frequency so high as to render the stimulation subthreshold (see fig. 5). The first response to this is a temporary fluctuation due to the heat of the stimulation. After allowing for this temperature equilibration it is obvious that the stimulation, in the average, has had no effect on the oxygen consumption. In the next period the frequency is reduced, but is still so high as to be subthreshold and the effect on the respiration is nil. When the frequency is further reduced, action potentials are produced and now for the first time the respiration is definitely increased. Figure 5 illustrates an experiment in which the frequency was changed six times each time holding the shock intensity constant. The composite group of curves

representing action potential, respiration, frequency of stimulation and intensity of stimulation show clearly the qualitative relationship between respiration and action potential and the complete lack of relationship between respiration and stimulatory energy; indeed, in this series of experiments there is an inverse relationship between respiration and energy of stimulation. It should be pointed out that the first and last periods of

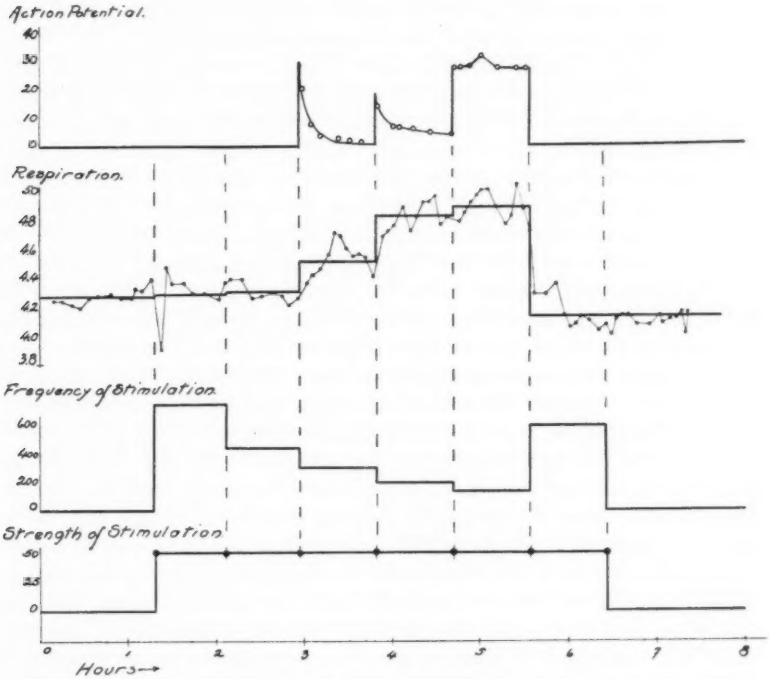

Fig. 5. Inverse relation between energy of simulation and oxygen consumption. May 23, 1932. Coördinates as before. Second line from bottom indicates frequency of stimulation in shocks per second. Weight of nerve, 20.2 mgm.; temperature 19.2°C.; bore of capillary, 0.416 mm.

stimulation were at frequencies of 725 and 575 per second respectively. In these cases the energy of the shocks is not strictly proportional to the frequency for the thyratron requires two sigmas for complete de-ionization. Hence any frequency greater than 500 per second will cause the intensity per shock to be somewhat lower than at rates below 500 per second. This does not alter the significance of the present experiments, however, for it simply means that the total energy of stimulation in these periods is

somewhat less than is indicated by the frequency. For the frequencies below 500 per second, the electrical stimulatory energy is directly proportional to the frequency; all other factors such as intensity, wave shape, etc., are constant. The factor of the amount and polarity of current spread from the stimulus is ruled out in this case for the intensity of stimulation and wave shape are maintained constant and therefore the escape should be constant regardless of the frequency, all other factors being unaltered. It is difficult to see how the results of this series of experiments could possibly be interpreted in terms of the Winterstein hypothesis.

Discussion. The distinction which Winterstein draws between *Reizung* and *Erregung*, so far as gaseous metabolism is concerned, is based mainly on experiments with nerves of European frogs. Gerard, Winterstein himself, and the writer agree, after having worked with both varieties, that the excess oxygen consumption following stimulation (*Reizung*) of the nerves of European frogs may be from five to ten times that resulting from stimulation of the nerves of American frogs. According to his own figures (Winterstein, 1930) the excess oxygen consumption of American bull frog nerves stimulated inside the respirometer may be as low as 11 per cent. We find an average increase of 15 per cent for American green frog sciatics stimulated about 125 times a second; this also expresses the average figure for the increase resulting from the stimulation of American bull frog motor roots. Winterstein concludes that whereas direct stimulation (*Reizung*) may cause the oxygen consumption to be increased as much as 77 per cent over the resting value, "physiological" stimulation (*Erregung*) produces *no* increase. Examination of his data shows, however, that in two cases, *Erregung* clearly produced increases of over 10 per cent and in two other cases (out of a total of five) increases of the same order of magnitude could not be ruled out because of the fact that the period of "physiological" stimulation always occurred early in the experiment when an enormous apparatus drift was clearly present. Hence we must conclude that regardless of whether nerve is stimulated inside or outside the vessel, there may be a small increase in metabolism which is associated with the impulse propagation, as the data above presented very clearly prove.

Winterstein believes that the large increases which result from *Reizung* are due to an unphysiological electrochemical change locally under the electrodes. With certain technical reservations already mentioned his data do clearly indicate some such phenomenon. This would receive confirmation also from the apparent relation between shock strength and amount of increased metabolism, previously reported by Winterstein and Hirschberg (1927) and by Ledebur (1927). The experiments reported above, however, unquestionably show that no such relationship between shock strength and excess metabolism exists in the case of Ameri-

can green frog sciatics. Nor is there any great distinction between *Reizung* and *Erregung* in the case of American bull frog sciatics, as Winterstein himself has shown.

There is no reason to believe that the structural or chemical differences between European and American nerves which produce this distinction between *Reizung* and *Erregung*—provided the distinction is not entirely due to technical imperfections—would involve factors fundamental to the mechanism of the propagation of the impulse. And in a theoretical discussion of *essential* mechanisms we must confine ourselves to limiting conditions. Therefore we must come to the conclusion that when a nerve actively conducts impulses its metabolism may be raised somewhat thereby, and that this rise is some function of the propagated disturbance and not of the electrical energy of stimulation.

The important question now arises as to whether we are justified in dealing with average figures, even in the case of our own experiments, when attempting to evaluate the essential nature of the propagated disturbance. We have found the problem of the absolute increase in oxygen consumption of stimulated nerve extremely difficult for technical reasons. Nevertheless, we have obtained satisfactory data from some of these experiments —the details will be published later—which indicate that bull frog motor roots may be stimulated at rates of about 50 shocks per second for extended periods without the slightest trace of any increase in oxygen consumption. Other experiments indicate that the excess oxygen consumption per impulse may not be greater at lower frequencies than at high, as is usually assumed; it may actually be zero.

To what, then, is this excess metabolism of activity due? Evidence is beginning to accumulate linking it with after-potential phenomena rather than with the spike processes. This evidence will be discussed in the succeeding paper in which it is shown that the excess metabolism per impulse is greatly increased in nerves in which the after-potential has been augmented by veratrinization. In still other preliminary experiments we have observed a rough temporal parallelism between the rate of increase of metabolism and the augmentation of the after-potential resulting from rapid stimulation.

It is entirely possible that data of this sort will eventually support the conclusion that the excess metabolism of activity of nerve—and possibly also the increased heat production—depends *entirely* upon late reactions such as are responsible for the after-potential. Stated in another way, it is possible that the propagation of spike potentials at frequencies low enough to avoid the development of large after-potentials may be associated with no increased metabolism whatever. And this may be the real meaning of the distinction which Winterstein draws between *Reizung* and *Erregung*. Considering Winterstein's double vessel experiments, how-

ever, if the large increases of metabolism associated with *Reizung* are actually reflected in large after-potentials, it is difficult to believe that stimulation outside the vessel (*Erregung*) would not give rise to similarly well developed but *propagated* after-potentials inside the vessel. In any event the difficult problem would still remain as to whether these accessory phenomena which may be the cause of the excess energy expenditure in rapidly stimulated nerves are artifacts due to experimental procedure or whether they do exist when nerve conveys impulses under strictly physiological conditions.

SUMMARY

A sensitive method is described for the simultaneous measurement of the oxygen consumption, electric response (spike and after-potential), strength, and frequency of stimulation.

The respirometer is a volumeter sensitive to change in volume of the order of 5×10^{-7} cc. In average good experiments it has been possible to measure the respiration of a 10 mgm. nerve (wet weight) with variations of less than 2 to 3 per cent for 2 minute readings. This accuracy has been made possible by the use of a thermostat specially designed to eliminate temperature pockets and to maintain the average temperature constant to less than 0.001°C. The electric response is measured oscillographically, the amplifier being capable of a voltage amplification of the order of 10^6. The stimulator is a thyratron device designed to give quantitatively controllable shocks at any frequency up to 500 per second.

A contribution is made to the controversy regarding the artificiality of the excess oxygen consumption which results from direct electrical stimulation of nerve. It can be shown by three distinct methods that the small increase (15 per cent) in respiration of American green frog sciatics and bull frog motor roots resulting from stimulation depends upon the production of action potentials and is independent of the electrical energy of the stimuli. It is pointed out that the absolute increase of respiration resulting from stimulation varies only qualitatively with spike height. The suggestion of Winterstein that the physiological propagation of the impulse is not associated with increased oxygen consumption is discussed.

I wish to record my appreciation of the willing coöperation of Mr. Otto H. A. Schmitt and of the efficient and patient services of Mr. Henry Breymann through the trying period of the development of this technique.

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THE RELATION BETWEEN THE AFTER-POTENTIAL AND OXIDATIVE PROCESSES IN MEDULLATED NERVE

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It has long been known that oxygen is required for the maintenance of normal nerve activity; but studies involving the effect of changes in the state of oxidation upon the processes occurring in functioning nerve have been, for the most part, confined to the propagated disturbance and its electrical sign, the spike. With the realization, which has developed in recent years, that the action potential is made up of two parts, the spike and the after-potential, the need for more extensive studies of the latter has become apparent.

Meyerhof and Schultz (1929), studying the oxygen uptake of leg nerves of *Maia squinado*, found that the resting rate of oxygen consumption, reckoned per gram dry weight, was ten times higher than in the case of frog sciatics, and that stimulation at the rate of 10 per second called forth an oxygen consumption, reckoned per gram dry weight per impulse, twenty times greater than frog sciatics. They offered the suggestion that this greatly increased metabolism resulting from stimulation was intimately connected with the large "retentions" found by Levin (1927) in similar material. Aside from this work the information available concerning the relation between the after-potential and oxidative processes has been derived from studies of the characteristics of the potential during and after asphyxia (Furusawa, 1929; Gerard, 1930a; Amberson, Parpart and Sanders, 1931). The experiments described in the present communication supply additional information on the latter problem, but they were devised primarily to answer two questions: Does an acceleration in the rate of utilization of oxygen occur during the progress of the after-potential? and, is the same respiratory catalyst necessary for the production of the after-potential which is necessary for the maintenance of the spike?

OXYGEN UTILIZATION DURING THE AFTER-POTENTIAL. The argument on which the solution of the first question is based is as follows. From the experiments of Amberson, Parpart and Sanders we know that oxygen is necessary for the after-potential, for the latter is more readily depressed in asphyxia than the spike itself. This fact taken alone, however, does

not differentiate between two possible interpretations: one, that there is an oxidation occurring as part of the after-potential process; the other, that oxidation is necessary in order to maintain the nerve in the proper condition for the production of an after-potential. For a decision between the two possibilities, simultaneous measurements of the metabolism and the after-potential are needed. If it could be shown that in two nerves producing a series of spikes, equivalent as to number and height, the rise of oxygen utilization over the resting rate is higher in the nerve displaying the larger after-potentials, there would be every reason to believe that the larger metabolism is part of the process occasioning the larger after-potential and presumably occurs during its course.

The means of performing the foregoing experiment is afforded by veratrinized nerve, for Graham (1930) has shown that veratrin properly applied increases the after-potential enormously without changing the spike.

Effect of veratrin on the resting metabolism of nerve. Before studying the effect of veratrin on the metabolism of activity, its effect on resting metabolism was investigated. For this purpose symmetrical nerves were dissected from four or five frogs, weighed and placed in Ringer's solution. One group of nerves was then painted with veratrin solution (veratrin hydrochloride, 1–50,000, dissolved in Ringer's solution and brought to a pH of 7.4) and placed in a moist chamber on wet filter paper. After 30 minutes to an hour the nerves were again painted just before being transferred to the respirometer vessel. The veratrinized and the control nerves were placed on the walls of their respective vessels in all but one instance without the addition of Ringer's solution, and their oxygen consumption measured. At the end of the experiments the nerves were withdrawn from the vessels and tested with the oscillograph to ascertain their ability to produce spikes and after-potentials. The after-potentials of the veratrinized nerves were found to be much higher than those of the control nerves.

Table 1 shows that veratrinizing nerves, in the manner described, has a slight stimulating action on the resting metabolism, amounting to an increase of about 15 per cent above the resting rate. In the instance in which the nerves were placed in the vessels in Ringer's solution (table 1, Oct. 20) the after-potentials were greatly decreased as compared with their original size, although an increase of metabolism still obtained.

An analysis of a typical experiment (Oct. 31) from table 1 with respect to the spikes and after-potentials of the individual nerves is shown in table 2. As recorded at the end of the metabolism measurements, the average spike height was found to be relatively little affected, whereas the average area under the after-potential curves (measured to about 300 sigmas only) was increased almost tenfold. The metabolism of the poisoned nerves was 18 per cent higher than that of the control nerves.

The effect of veratrin on the oxygen consumption of stimulated nerves. The plan of experimentation adopted was based on the assumption that, if large after-potentials occasion a sufficient increase in metabolism, it would be possible to demonstrate a definitely increased oxygen consumption by

TABLE 1

DATE	TEMPERATURE	NUMBER OF NERVES	O_2 IN CU. MM. PER GRAM WET WEIGHT PER HOUR		INCREASE	TYPE OF RESPIROMETER
			Control	Veratrinized		
1931	°C.				*per cent*	
June 19	20.9	4	42.6	50.7	19	Differential volumeters
	20.9	4	43.3	55.9	29	Differential volumeters
June 26	20.9	4	37.8	42.7	13	Differential volumeters
June 22	20.9	4	41.3	46.2	12	Differential volumeters
	20.9	4	36.1	39.9	11	Differential volumeters
1932						
Oct. 24	22.4	5	30.7	28.6	−7	Simple manometers
Oct. 28	21.1	4	41.5	57.3	27	Differential manometers
Oct. 31	18.9	4	29.8	35.2	18	Differential manometers
Oct. 20*	22.7	4	59.5	67.8	14	Differential manometers

* Nerves placed in 1 cc. of Ringer's solution in the vessel.

TABLE 2

CONTROL NERVES			VERATRINIZED NERVES				
Spike height	Area under after-potential	Oxygen consumption	Spike height	Area under after-potential	Oxygen consumption	Increase over control nerves	
						After-potential	Oxygen consumption
*mm.**			*mm.**			*per cent*	*per cent*
67	17		40	276			
13	7		36	323			
23	21		40	540			
80	104		36	401			
Average 46	37	29.8	38	385	35.2	940	18

* 1 mm. equals 0.125 mv.

veratrinized nerves at rates of stimulation so low that no increase could be detected in an unpoisoned nerve similarly treated.

Now while in occasional experiments we do observe excess oxygen consumptions of 30 to 40 per cent resulting from rapid stimulation of unpoisoned nerves, the data from numerous experiments performed in the manner previously described (Schmitt, 1932) indicate that the average

increase is of the order of 15 per cent, when the rate of stimulation is about 125 per second. Hence it is reasonable to suppose that if the rate of stimulation were decreased to 2 per second the excess oxygen consumption would be less than 1 per cent, an amount too low to be detected with accuracy (owing to the possibility of drift), even over periods of 30 to 60 minutes' duration.

The first step was to perform a series of experiments upon unpoisoned nerves using low rates of stimulation. The method used for this purpose was similar to that already described (Schmitt, 1932), except that extreme precaution was necessary to avoid apparatus drift and factors which caused irregularities of movement of the droplet of kerosene in the capillary. When operating with these precautions it was possible to maintain such a steady base line that variations in the metabolic rate occurring over long periods were significant. The nerves used in the experiments showed a persistent dropping off in the rate of respiration over a period of hours, and this we concluded must have been due to real metabolic changes.

The nerve was prepared in the manner already described, the veratrin being painted on twice during a period of an hour. In most of the experiments the end of the nerve was heated to facilitate oscillographic recording of the after-potential. Although this heating had surprisingly little effect on the regularity of the curve of oxygen uptake, a few experiments were done without heating, as a control. After a period of rest the nerve was stimulated with a shock maximal for the spike of the *A* fibers and at a frequency of about 2 shocks per second. The spike height was recorded and the shape of the after-potential traced directly on the face of the tube with a wax pencil, to be copied immediately on tracing paper as a permanent record. In the present experiments, owing to the enormous increase in the after-potentials caused by the veratrin, this method was sufficiently accurate, and photographic recording was usually omitted. Figure 1 is typical of the results of such experiments on unpoisoned nerves. It is obvious that the periods of slow stimulation have little or no effect on the oxygen uptake of the nerve. After three or four periods of slow stimulation it was customary to test the effect of rapid stimulation in order to demonstrate that the nerve was capable of giving the increase in metabolism usual at the higher rate. In the experiment plotted in figure 1 the terminal period of rapid stimulation resulted in an increase of about 10 per cent over the resting rate.

After having satisfied ourselves that unpoisoned nerves show no appreciable excess oxygen consumption at a frequency of 2 shocks per second, we next performed a similar series of experiments in which the nerve had been previously veratrinized to increase the after-potential. This series of experiments showed a decided increase in oxygen consumption amounting in some instances to over 10 per cent of the resting rate. Figure 1 illus-

trates such an experiment. As a rule the spike potential remained fairly constant throughout the experiment, seldom decreasing more than 10 per cent of its original height. The after-potential likewise was fairly constant but, if anything, tended to increase somewhat during the course of the

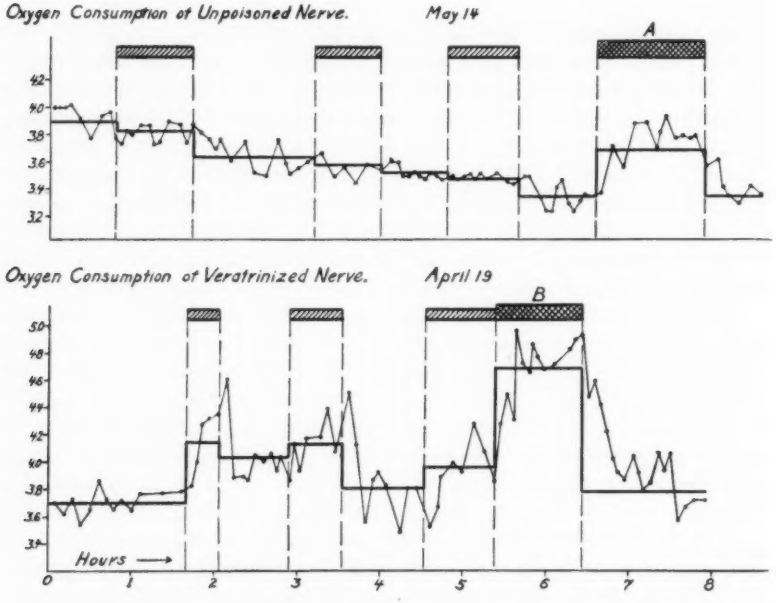

Fig. 1. The effect of stimulation at the rate of two shocks per second on the oxygen consumption of normal nerve (May 14) and of veratrinized nerve (April 19). Ordinates represent rate of respiration in divisions per minute (1 division = 0.0125 mm.; capillary bore equals 0.4 mm.; nerve wet weights equal 14.6 and 23.9 mm., respectively); abscissae represent time in hours. Points are actual rate readings, horizontal heavy lines show the average rates of respiration for the several periods. Periods of stimulation marked by rectangles with barred tops. Where the tops of the rectangles are cross-hatched (*A* and *B*) stimulation was at the rate of 110 shocks per second.

experiment. In this case the terminal period of rapid stimulation resulted in an increase of about 25 per cent over the resting rate.

The foregoing experiments were made possible by the development of the sensitive method described in the preceding paper, which permits simultaneous recording of spike and after-potential and oxygen consumption. In the spring of 1931, during the period in which this technique was being developed, a series of perhaps 25 experiments was performed, using very small Warburg respirometers of about 1.5 cc. volume, containing two platinum electrodes and an inset for alkali. The experiments

were so planned that the control nerve was placed in a vessel on one differential manometer while the veratrinized nerve was placed in a vessel on another differential manometer. The wires were arranged so that the nerves would be stimulated in series with each other and also in series with a nerve-muscle preparation outside the vessel. Since it was impossible to record the action potentials in these experiments, this arrangement was adopted in an attempt to regulate the strength of shock to a just maximal value and to stimulate both control and experimental nerves with approximately the same strength of shock. The strength of the induction shocks was adjusted to a value such that the external nerve muscle preparation would receive a just maximal shock. Occasionally, in this series of experiments, in spite of the low rate of stimulation, even the control nerve would show an increase of 10 to 15 per cent over the resting rate, but in all of the experiments the excess oxygen consumption of stimulated veratrinized nerves exceeded that of the control nerves. In one extreme case the veratrinized nerve showed a 300 per cent increase on stimulation at the rate of 2 shocks per second. Withdrawn from the vessel at the end of the experiment, this veratrinized nerve showed very large after-potentials as compared with the control nerve. The results of this preliminary series of experiments indicated that slow stimulation of veratrinized nerves caused an average increase in metabolism equal to something like 50 per cent over the resting metabolism and that similar stimulation of unpoisoned nerves usually led to only small increases, although in several cases the increase amounted to 10 to 20 per cent of the resting rate. However, in view of the insensitivity of the method, the ever present apparatus drift, and the complete lack of control over the action potential, the experiments were discontinued pending the development of the improved technique described above.

The effect of carbon monoxide on the spike and after-potential. It was shown by Schmitt (1930) that nerve contains a catalyst similar to the respiratory enzyme of Warburg and that the ability of the nerve to respire normally and to conduct the action potential depends upon the proper functioning of this catalyst. The proof rested upon the demonstration that nerve can be poisoned by carbon monoxide and restored by the action of light. In the absence of spectroscopic data it was impossible to identify the catalyst with the respiratory enzyme of Warburg, but, in view of its sensitivity to cyanide, hydrogen sulphide and carbon monoxide, it is safe to assume that the catalyst is probably a hemin compound having to do with the promotion of nerve oxidation.

In order to determine whether the same catalyst is connected with the bursts of oxidation associated with after-potentials, nerves were subjected to carbon monoxide according to a method similar to the one previously employed, the only difference being that provision was made for the recording of the spike and after-potentials during the course of the experiments. Measurements of the spike height and the records of the after-potential, which involve different amplifications, were made as nearly simultaneously as possible so that the rapid changes which take place during and immediately following illumination would not prevent a comparison of the two quantities.

The course of a typical experiment is shown in figure 2. At the time at

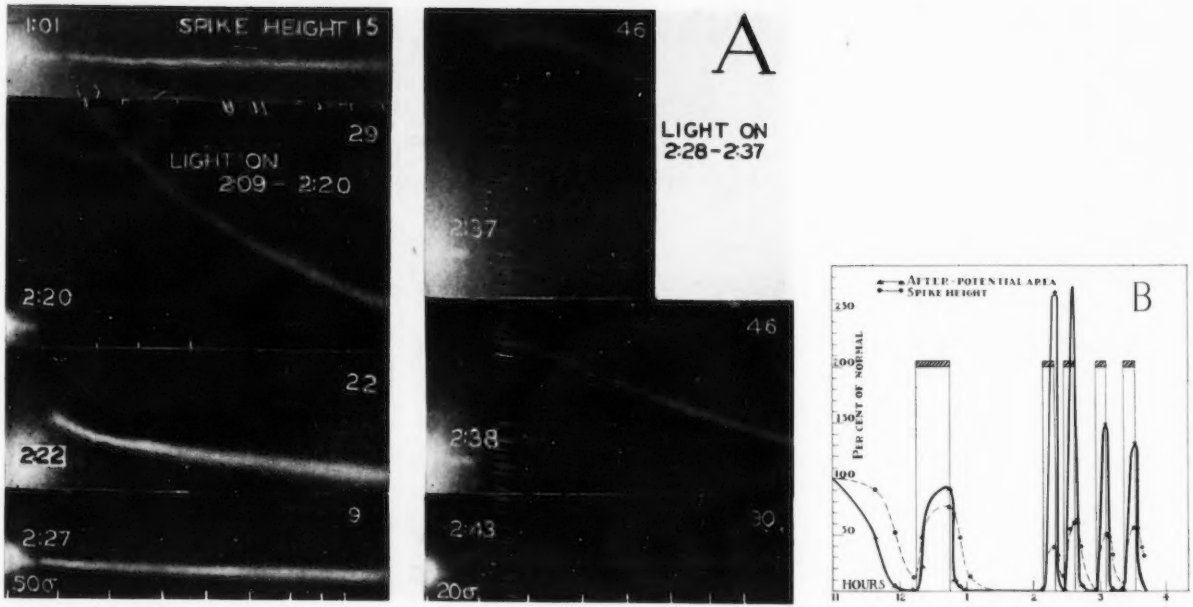

Fig. 2. Effect of carbon monoxide on nerve potentials. The nerve was placed in a mixture of 95.8 per cent CO and 4.2 per cent O_2 at start of experiment. *A*, photographic records showing the rapid disappearance of the after-potential upon turning off illumination. The times at which the records were made are indicated at the lower left hand corner; the spike heights are shown at upper right hand corner. *B*, graph showing the effect on the spike height (points) and on the after-potential area (triangles). Ordinates, spike height and area under after-potentials (measured to 350 sigmas only) in per cent of the steady values obtaining before passing in carbon monoxide; abscissae, time in hours. Periods of illumination marked as in figure 1.

which the first record of part A was taken, the carbon monoxide-oxygen mixture had reduced the after-potential to a value so small as not to be detectable at the amplification used (1 mv. equals 20 cm.), but the spike (not visible in the record) was still 35 per cent of its original height. Although the spike was more resistant than the after-potential, it too was later reduced to zero. Intense illumination was then applied and both the spike and the after-potential reappeared and rose rapidly in height, the after-potential rising more rapidly than the spike and reaching a value

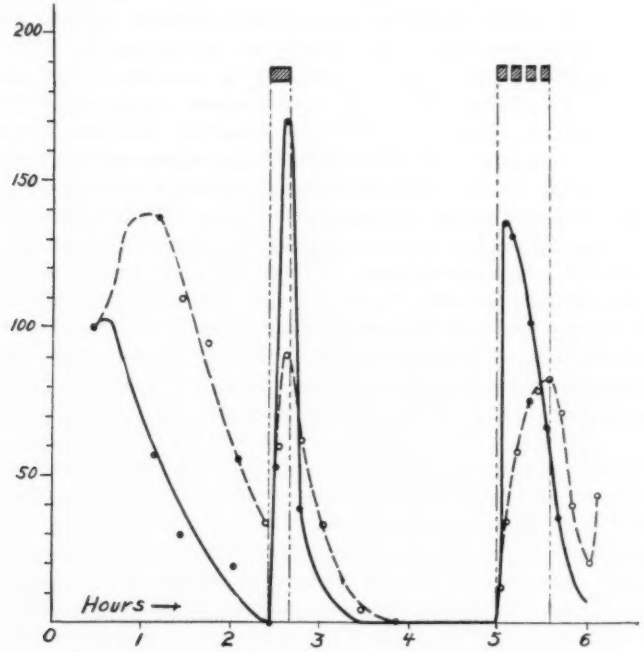

Fig. 3. Effect of carbon monoxide on spike and after-potentials. Coördinates and gas mixture as in figure 2.

considerably higher than its initial value (second illumination of fig. 2 B). Discontinuation of illumination caused an immediate decline of both potentials, the after-potential again going to apparent extinction considerably ahead of the spike. Another illumination now permitted the cycle to be repeated. The results obtained in a succession of illuminations are shown in part B of figure 2. The rapid decline in the after-potential when the illumination is extinguished is apparent and reveals the necessity for working as rapidly as possible in order to get the points on the curve. As it

were, one actually turns off the after-potential when one turns off the illumination.

In most of the experiments the period of illumination was brief and, while the spike height was determined every few minutes during the period of illumination, no photographs of the after-potential were made until the moment after the light had been turned off. This was assumed to represent the maximum recovery of the after-potential during the period of illumination. In one experiment, however, we purposely set out to see whether the after-potential would remain at a higher level throughout the period of illumination or whether it would again decline. This experiment was accomplished by occasionally turning off the arc light for a period of about 2 minutes, which is the time necessary for the room to become completely dark for photography of the after-potential wave. From figure 3 it is seen that the 35-minute period of illumination was interrupted three times in this manner. It is also seen that the after-potential reached its maximum height within 5 minutes after the beginning of the illumination; thereafter each succeeding reading revealed that the after-potential was falling rather rapidly, until at the end of illumination it had sunk to a value 60 per cent of its original amount. During this same period, however, the spike potential was steadily increasing and it is doubtful, even in this particular case, whether it had reached its maximum when the illumination was discontinued. This behavior again indicates the differential response of the two potentials to experimental conditions.

Discussion. Out of the foregoing experiments the answers to the two questions originally posed emerge with some clearness. The first question, as to the rate of utilization of oxygen during the after-potential, is answered by the observation that when a series of spikes is associated with large after-potentials the latter are immediately connected with an oxidation which would not occur if spikes of the same number and magnitude were produced in fresh normal nerve. Veratrinized nerve shows a 10 per cent increase in metabolism at 2 spikes per second, while it takes 100 similar spikes to produce an equivalent metabolic increase in unpoisoned nerve. The second question, as to whether the spike and after-potential are maintained by the same respiratory catalyst, is answered by the fact that both can be extinguished by carbon monoxide and restored by strong illumination.

The experiments with carbon monoxide bring out the same changes which are caused by asphyxia. There is the same period of asphyxial depression of the after-potential and the same over-shooting during a period of illumination as is seen immediately after restoration of oxygen to a nerve asphyxiated in nitrogen. Furthermore, during continued illumination the after-potential tends to return toward a normal value, just as it does after a sufficient recovery period in oxygen. A minor differ-

ence occurs, but it is not one which negates the significance of the general parallelism. During continuous illumination the spike potential may drop to complete extinction (Schmitt, 1930) and even before the decline of the spike the after-potential may become subnormal. This latter difference does not manifest itself in all experiments and its cause is not clear.

The disappearance of the after-potential in asphyxiated nerve might be due to an alteration in the state of the nerve resulting from continued lack of oxidation and not from a lack of oxidizing material at the time the after-potential should occur; but even if this possible interpretation should prove to be the correct one, the veratrin experiments show that the whole of the relationship of oxygen to the after-potential would not be thereby described, for veratrinized nerve, which in the resting state has plenty of available oxygen and uses it at a rate greater than normal, increases the utilization in association with the after-potentials occurring during activity. Whether or not the oxidation occurs in exact parallelism with the potential cannot be decided from the present data. Figure 1 shows a delay with respect to the period of stimulation of both the rise and decline of the curve of excess oxygen consumption; but this delay may be explained by a physical time lag for gaseous equilibration in the nerve and for temperature equilibration necessitated by the heat liberated by the shocks.

About the more intimate nature of the relationship of the after-potential to oxidation no statement can be made as there are no crucial experiments which permit a decision even with regard to such a fundamental question as whether a large after-potential is the cause of a large oxidation or is occasioned by it.

Aside from their bearing upon the nature of the after-potential the veratrin experiments are of interest from the standpoint of veratrin poisoning as such. A nerve so treated is subject to a continuous waste of energy. Even at rest the metabolism is in the average about 15 per cent above normal; and activity is only possible with an additional waste, since each spike is attended by an abnormally large metabolism.

SUMMARY

The effect of veratrin upon the metabolism of the sciatic nerve of the frog, *Rana pipiens*, was studied during rest and activity.

The oxygen consumption of resting veratrinized nerves was about 15 per cent greater than that of unpoisoned paired nerves kept as a control.

The oxygen consumption of single nerves was measured in a sensitive volumeter when the nerves were stimulated with shocks just strong enough to give maximal *A* spikes, the nerve responses being at all times controlled by observations of the spike and after-potentials with an oscillograph. Normal nerves showed no definite increase in oxygen utilization when stimulated twice per second, but veratrinized nerves under the same condi-

tions showed an increase of 5 to 15 per cent above the resting rate. The responses of the veratrinized nerves were characterized by very large after-potentials.

Carbon monoxide depresses the after-potential in nerve more rapidly than it does the spike. Illumination causes the after-potential to rise very much more rapidly than the spike, but this rise is temporary and, after exceeding the normal value, the after-potential may recede considerably below the initial level even during illumination.

It is concluded that the passage of a spike along a nerve initiates a burst of oxidation which must be associated with the processes of the after-potential. This oxidation is mediated by a hemin-like catalytic system which can be poisoned by carbon monoxide and which is at least partially photosensitive.

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UTERINE MOTILITY IN HYPOPHYSECTOMIZED AND IN PREGNANT RABBITS[1]

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Recently Reynolds and Allen (1932) have shown that spontaneous uterine motility or that which may be induced by the oestrogenic (follicular) hormone (Theelin) can be effectively inhibited by small amounts of the active corpus luteum extract (Progestin) prepared by Allen and Corner (1929). It had previously been shown that the response of rhythmical contractions of a normal rabbit's uterus to the oestrogenic hormone is constant and that in its absence, quiescence inevitably supervenes (Reynolds, 1931a, 1932, 1932a). Utilizing these facts, we studied the uterine motility of hypophysectomized and pregnant rabbits. In the first group of experiments an effort was made to determine whether or not the corpora lutea of pregnancy lose their ability to counteract the effect of Theelin on uterine motility following hypophysectomy, and so in this respect cease to be functional. The second series of experiments were planned to determine the period of pregnancy at which the corpora lutea cease to inhibit the Theelin response by obtaining motility records directly from gravid uteri in the unanesthetized rabbit. These experiments were performed, therefore, under circumstances in which the hormonal and morphological conditions were essentially normal and should tend to reveal the time in gestation in this species at which the functional activity of the corpora lutea may be said to have waned as regards uterine motility.

Hypophysectomy and uterine motility (fig. 1). It has been shown by Firor (1933) that when rabbits are hypophysectomized on the third day *post coitum* the corpora lutea retrogress. To ascertain whether or not these anatomical relations are associated with an equally demonstrable functional deficiency was the object of this group of experiments. Six rabbits comprise the series. In five of them the hypophysis was removed. This fact was confirmed in each instance by serial sections of the postopera-

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tive contents of the sella. The remaining rabbit served as an operative control. All rabbits were *post partum* does. They were kept in separate cages and mated on only one day. The protocols of these experiments are as follows:

No. 103. Weight 1.6 kgm.
May 26, 1932: Coitus.
May 30: Laparotomy to confirm the presence of fresh corpora lutea. Uterine fistula made and hypophysectomy performed.
June 2: Uterine motility recorded. The uterus was slightly irritable as evidenced by small irregular contractions, which varied in frequency and in intensity. Theelin was injected intravenously in equal amounts at 11 a.m., 5 p.m., and 9 p.m. The total dosage of Theelin equalled 10 rat units per kilo of body weight.
10 p.m.: A record of uterine motility was made and showed large rhythmical contractions, a typical oestrin response.
June 3: 10 a.m A second record confirmed the one taken 13 hours before. (Cf. Reynolds, 1931a, for typical Theelin response.)
June 6: Autopsy: Uterus in healthy condition. Ovaries showed regressing corpora lutea. Sella grossly empty. Serial sections subsequently confirmed the complete absence of all hypophyseal tissue.
No. 104. The operations, the injections, the time relationships, and the records are an exact duplicate of those of the preceding rabbit.
No. 113. Weight 1.6 kgm. (See fig. 1.)
June 4: Coitus.
June 7: Laparotomy. Had ovulated; fistula prepared and hypophysectomy performed.
June 9: Normal motility. Slightly irritable uterus. Theelin injected in three doses, 4 hours apart. Total amount equalled 5 rat units per kilo.
Nine hours after first injection, typical oestrin-response.
June 10: Twenty-five hours later, typical oestrin motility. The records of this rabbit are reproduced in figure 1.
No. 119. Same in all respects as 113 except fistula and hypophysectomy were performed on the fourth day after coitus instead of on the third day and that the animal was sacrificed when the second record was completed, 25 hours after the first injection of Theelin.
Typical oestrin-response. Autopsy: Genital tract in perfect condition. No infection. Ovaries small. No large corpora lutea.
No. 117. Weight 2.5 kgm. In all respects similar to 119.
July 2: Sacrificed. No gross corpora; perfect genital tract.
No. 75. Control. Weight 3.0 kgm. (See fig. 1.)
June 4: Coitus.
June 7: Laparotomy: Recent ovulation; fistula prepared and hypophysectomy attempted.
June 9: Normal motility; slight irregular contractions. Theelin injected intravenously three times at 4-hour intervals. Total injection equivalent to 5 rat units per kilo.
Nine and a half hours after first injection: Only slight irregular contractions, identical with the normal record obtained before Theelin was injected.

June 10: Similar record as on June 9. The rabbit then received three injections of Theelin, totalling 150 rat units.
Nine hours later: Records identical with previous records.
Twenty hours later: Quiescent uterus. No oestrin-response.
June 11: Autopsy: Sella full; hypophysis intact. Gravid uterus; ovaries with large corpora lutea.
The records of this rabbit are reproduced in figure 1.

This group of experiments shows clearly that within *forty-eight* hours after hypophysectomy the corpora lutea of five days' duration are ineffective in preventing typical oestrin motility in the rabbit's uterus. The thorough-

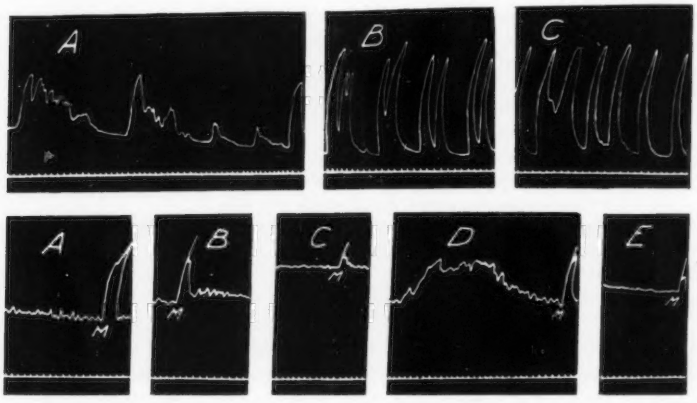

Fig. 1. Top: Uterine motility record of no. 113: A, before Theelin; B, nine hours after Theelin; C, twenty-five hours after Theelin. Lower: Record of no. 75: A, before Theelin; B, nine hours after Theelin; C, twenty-five hours after Theelin; D, nine hours after second injection of Theelin; E, twenty hours after second injection. *M*, mechanical response.

ness of this loss of inhibitory power of the corpora lutea is emphasized by the fact that minute doses (5 rat units per kilo) of Theelin sufficed to produce typical oestrin motility, whereas in the control animal doses ten times as great were ineffectual. One cannot claim, of course, that the loss of one function of the corpora is indisputable evidence of the loss of other functions, but the findings are in complete agreement with the histological alterations of the corpora lutea which one finds following hypophysectomy. Suggestive evidence has been advanced elsewhere, moreover, that the uterine motility-affecting and the proliferating hormone (Progestin) are identical (Reynolds and Allen, 1932).

Uterine motility in pregnant rabbits. The second series of experiments

consisted in measuring uterine motility in rabbits on the 15th–16th, 21st, 25th, and 27th days of normal pregnancy. In the first mentioned period (15th or 16th day of gestation) eight rabbits were tested. The protocol of one is given in full.

No. 91. Weight 2.3 kgm.
May 31: Coitus.
June 14: Laparotomy confirming pregnancy. Fistula made.
June 15: No spontaneous uterine motility. 11:15 a.m., 2:30 p.m., 4:30 p.m.: Theelin 160 rat units injected at each period. (Total in three injections of 200 rat units per kilo of body weight.)
9:45 p.m. Ten hours after first injection. No motility; good mechanical response, showing patency of system.
June 16: 10:30 a.m. Twenty-three hours after first injection: No motility; good mechanical response.
June 16: Laparotomy: Uterus healthy; living fetuses.
No. 93. Identical with 91 except dosage of 150 rat units Theelin per kilo.
No. 114. Identical with 91.
No. 126. Sixteenth day of pregnancy: 180 rat units per kilo. Nine hours: no Theelin response. Twenty-four hours: no Theelin response.
No. 92. Identical with 91 except 60 rat units per kilo.
No. 123. Sixteenth day of pregnancy: 200 rat units per kilo. Eight and a half hours: no response.
No. 127. Identical with 91.
No. 128. Same as 91.

It seems clear, therefore, that in mid-pregnancy the corpora lutea are sufficiently active to inhibit the uterine motility normally induced with moderate doses of Theelin. In fact, from six to forty times the usually effective dose of Theelin for the castrate rabbit was given, but there was no evidence whatsoever of oestrin motility.

In the 21-day series seven rabbits were tested. A protocol of a typical experiment follows:

No. 116. Weight 3.0 kgm.
May 31: Coitus.
June 20: Laparotomy. Pregnancy confirmed and fistula prepared.
June 21: Normal record showed quiescent uterus. 11 a.m., 2 p.m., 5 p.m.: injected with 200 rat units of Theelin. The total dosage was equivalent to 200 rat units per kilo.
10 p.m., eleven hours after first injection: Slight motility, irregular.
June 22: Uterus quiescent. No motility.
Autopsy: Uterus perfectly healthy.

The other rabbits in this series were similar to 116 except as indicated in the following notes:

No. 115. First record made 8½ hours after first Theelin injection showed slight irregular motility.

No. 71. Was given 100 rat units Theelin per kilo.
No. 149. Was given only 10 rat units Theelin per kilo.
No. 147. Was given only 10 rat units per kilo. Records taken 6½ and 12 hours after first injection of Theelin: Both showed quiescent uterus.
No. 148. Same as 147.
No. 151. Same as 147.

These seven experiments are in close agreement. They show that on the 22nd day of pregnancy in rabbits the corpora lutea are functional to the point of inhibiting the motility produced by Theelin injections. No rabbit in any test between the 6th and 20th hours after injection with Theelin showed a typical Theelin response, and this was true despite injections varying from the normally effective amount to twenty times the amount of Theelin needed to cause rhythmical motility in castrate rabbits.

In the 25-day series there were four rabbits, each of which was given 10 rat units per kilo of Theelin intravenously. Records of motility were

TABLE 1

Summarizing table of data of effect of Theelin on uterine motility at various stages of pregnancy in the rabbit

DAY OF PREGNANCY: POST COITUM	AMOUNT THEELIN	NUMBER OF RABBITS SHOWING		TOTAL RABBITS
		Non-effect of Theelin on uterus	Positive effect of Theelin on uterus	
	r.u./kilo			
15th–16th	60–200	8	0	8
21st–22nd	10–200	7	0	7
25th–26th	10	4	0	4
27th–28th	2	0	2	2

taken from 5½ to 16 hours after the first injection, and in not one case was typical oestrin-response elicited. In all of the 25-day rabbits the records which were made before the injections were begun showed an irritable uterus with spontaneous irregular contractions of moderate amplitude. Following the Theelin these contractions persisted for 5 to 6 hours and on the whole may have been of slightly greater force. After 9 hours, however, the uteri were entirely quiescent.

One rabbit was tested on the 27th day of pregnancy, and before any injections were made the motility record of the normally existing uterine contractions showed large rhythmical waves occurring regularly every 60 seconds. A single injection of 0.1 cc. of Theelin was administered. This was equivalent to about 2 rat units per kilo. The rabbit aborted one fetus through the fistula and died within 2 hours.

A healthy rabbit, 28 days pregnant, was given a similar dose and also aborted and died.

DISCUSSION. *Uterine motility following hypophysectomy.* The experiments of this group reveal two interesting points which indicate the nature of the hormonal changes occurring as a consequence of hypophysectomy during the early stage of pregnancy or pseudopregnancy. They show first, that within forty-eight hours following removal of the hypophysis, the motility-inhibiting function of the corpora lutea has completely waned. This effect is even more abrupt than the purely histological regressive changes of such corpora lutea would indicate (Firor, 1933; Smith and White, 1931). That the inhibiting influence of these structures is lost is shown by the fact that the amounts of Theelin employed here to induce motility are likewise liminal for uterine motility in castrated rabbits (Reynolds, 1931a). Smaller quantities, therefore, would have revealed nothing. Moreover, functional corpora lutea (Reynolds, 1931b) and the active extracts from them (Reynolds and Allen, 1932) have been found to inhibit up to five hundred times this liminal quantity of Theelin.

The second point of interest is apparent from a consideration of the first. The records of spontaneous uterine motility, prior to administration of Theelin, reveal uteri which are quiescent, or which show some irregular motility of a sort that may be seen a day or so following castration, or such as occurs during the waning of Theelin-induced motility. The inference is, therefore, that as regards uterine motility, hypophysectomy results in a functional castration, as distinguished from operative castration. Thus the present results are in consonance with the histological findings on the rat, rabbit, and dog (Cf. E. Allen, 1932, p. 734; also Firor, 1933).

Uterine motility during pregnancy. Some objection may be raised concerning the validity of the data of this series on the grounds that to prepare a uterine fistula with a gravid uterus in the latter half of pregnancy would have profound and dire effects upon the course of gestation. Such a criticism is probably unfounded in view of the following considerations: None of these animals were allowed to carry their young to term, but all were sacrificed at the end of the experiment; that is to say, within two to three days following the operation. At this time they were found to have healthy uteri and living fetuses. This fact was true whether the uteri remained quiescent even after the injection of Theelin (15- to 21-day series) or whether there was some moderate degree of spontaneous motility (25- to 26-day series). Only the 27- and 28-day rabbits suffered ill consequences following the injection of Theelin. Up to the time of this injection each of these does appeared healthy and ate well. Clearly, the fistulae *per se* had no ill effects, else probably the whole series would have shown similar responses. As a matter of fact, the uteri of the 15- and 21-day rabbits were unaffected even after the experimental procedures, and were quiescent at all observed times.

Therefore, one may safely conclude from the data obtained in this series

that the corpus luteum is functioning at a high level of activity, as regards its influence on uterine musculature, through the twenty-first to the twenty-second day of gestation. The small degree of spontaneous motility seen on days 25 and 26 would seem to indicate a mild waning effect of the corpus luteum inasmuch as active Progestin-containing extracts retard spontaneous motility (Reynolds and Allen, 1932). Nevertheless, Theelin did not augment this motility. These instances parallel three cases reported elsewhere (Reynolds, 1931b) in which Theelin administered several days before the return of marked motility at the end of pseudopregnancy was without effect, despite, in one instance, the presence of feeble motility at the time. That this inhibitory action was due to the corpora lutea is indicated by the fact that their removal by castration cancelled at the same time the motility-inhibiting influence on the uterine musculature. Whether or not the spontaneous contractions observed in the present experiments represent a motility that is a physiological attribute of this stage of pregnancy or motility which is aggravated by virtue of the abnormal position of the uterus in the presence of a heightening irritability of the uterus at this time, cannot be said. In either event, it is significant that the fistulae of rabbits several days earlier in gestation do not show this motility, whereas within the space of two more days administration of 2 rat units of Theelin per kilogram of body weight results in abortion and death. The conclusion logically follows, we feel, that the protective influence of the corpus luteum upon the gravid uterus has by this time (27th day) been completely spent.

Knaus, using isolated uteri from sterile horns of unilateral pregnancies, finds them refractory to posterior pituitary extract on the 17th day of gestation, whereas from the 18th day diminishing amounts of posterior pituitary extracts elicit motility, with maximum responsiveness of the uterine strip about the time of parturition (Knaus, 1929, 1931a, b, c). It is apparent that the results obtained by this investigator, while in general similar to ours, do differ in that we find *no* evidence of *loss* of lutein function until about the middle of the fourth week and *complete loss* of lutein function as regards uterine motility by the end of the fourth week. Any attempted explanation of these divergent results would be speculative at the present time.

Factors affecting uterine motility at term. The present results are not incompatible with the view, recently expressed by Jeffcoate (1932), that oestrin is the predisposing hormonal agent responsible for the normal onset of labor. It is well known that in lower animals the last few days of pregnancy are associated with growth in the ovary of new follicles *pari passu* with the suspected decline of lutein function, now indicated in our experiments. Soon after parturition, if not allowed to suckle their young, these forms come into oestrus.

The situation in the human is not so clear, since the corpus luteum may be excised relatively early in gestation (cf. Jeffcoate, 1932; also E. Allen, 1932, pp. 501, 528). Nevertheless, it is known that at the time of parturition in the human the amount of oestrin is greater than at any other time under physiological conditions (Zondek, 1931; Runge and Clausnitzer, 1932; Runge, Hartmann and Sievers, 1932). The placenta is the probable site of secretion of this hormone (Allen, 1932, pp. 438, 463). It is not impossible that the anterior pituitary-like element present in the blood of pregnant women has a stabilizing action on the uterus and thus counteracts the presence of oestrin, since it has been shown to have such an effect on uterine motility in rabbits (Reynolds, 1932b, c). Also, Smith claims to have used it to check threatened abortion in women (G v.S. Smith, 1931) in doses so small it could barely have affected the ovary. Another possible alternative is that the placenta may elaborate Progestin, although it has never been found there (W. M. Allen, personal communication).

It is hardly necessary to point out that this view of the rôle of oestrin in relation to labor as expressed by Jeffcoate does not imply a synergistic action with oxytocin as recent reports suggest (Heller and Holtz, 1932; Marrian and Newton, 1932), for the best evidence does not support this opinion. Jeffcoate (1932) in controlled experiments demonstrated the fallacy of this view originally expressed by Bourne and Burn (1928). Smith (1932), moreover, has directly shown the non-essentiality of the posterior hypophysis for labor in rats, and van Dyke, Bailey and Bucy (1929) fail to find any oxytocic action of human cerebrospinal fluid when carefully tested on isolated uteri. Friedman and Friedman (1932) also fail to find evidence of oxytocin in cerebrospinal fluid from women and dogs when tested on the uterine fistula in unanesthetized rabbits. Furthermore, one of us has found that oestrin is the only substance yet found which makes the quiescent non-gravid uterus *in situ* contract (Reynolds, 1931a), and that whole posterior pituitary extracts and oxytocin mediate their effects only upon the already rhythmically contracting uterus *in situ* (Reynolds, 1930).

The variable span of gestation in the rabbit (Hammond, 1925), the human (Mandelstamm and Tschaikowsky, 1932), the monkey (Hartman, 1928), and other forms is additional but circumstantial evidence that an increasing irritability of the gravid uterus, indicated in our experiments and in those of Knaus above, may be associated with the increasing elaboration of the follicular hormone. This, in conjunction perhaps with intra-uterine or other changes, may be a predisposing factor in the normal onset of labor.

CONCLUSIONS

The above experiments show 1, that in rabbits hypophysectomized on the 5th to 6th day of pregnancy or pseudopregnancy, the corpora lutea

lose within 48 hours their power to inhibit typical uterine contractions induced by oestrin (Theelin); 2, that in unanesthetized pregnant rabbits the uterus is refractory to the motility-inducing action of Theelin until the 26th day of pregnancy, but on the 27th day the injection of Theelin leads to abortion and death; 3, that the corpus luteum functions at a high level, as regards maintaining uterine quiescence, until about the middle of the fourth week of gestation, and is no longer functioning in this regard by the end of the fourth week.

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STUDIES ON THE PHYSIOLOGY OF SLEEP

VII. The Effect of the Position of the Body and of Sleep on Rectal Temperature in Man

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It is a well-known fact that the temperature of homoiothermal animals is really not constant, but shows fluctuations of one or more degrees under different conditions Muscular activity produces a temporary rise in body temperature which may be subjectively felt. The most regular change in temperature is associated with the twenty-four hour period of day and night, most individuals showing a well-marked diurnal temperature curve with a maximum in the afternoon and a minimum in the early hours of the morning. The fact that one's temperature begins to fall long before bedtime and follows its usual curve if one stays up all night has been considered by some as showing that sleep is not directly responsible for the low night temperature. Observations made by Piéron (1913) on several subjects by discontinuous readings and continuous registration led him to conclude that neither the onset of sleep nor awakening has any direct effect upon body temperature, provided the person lies quietly in bed. On the other hand, he points out that night-birds have a higher temperature at night than in the daytime, and the night-workers often show an inverted temperature curve. Galbraith and Simpson (1904) reported that the diurnal temperature curve disappeared in monkeys kept in the dark, and Kleitman (1923) noted a tendency of the diurnal temperature curve to flatten out upon prolonged wakefulness. Pembrey and Nicol (1899) also considered rest and sleep as essential factors in the nightly temperature drop. They found that the same individuals at the same hours of the day had a lower temperature if they were lying down than if they were up. They observed a rise in temperature upon getting up and dressing in the morning (0.28 to 0.44° Fahrenheit), and a smaller rise upon awakening and remaining in bed.

We reinvestigated the effect of position of the body and of sleep upon rectal temperature by the use of an electrical resistance recording thermometer (made by Leeds and Northrup) which permitted us to read the temperature with an accuracy of 0.02° Fahrenheit. Four young male adults were the

subjects of this investigation, each undergoing a number of tests, following one of three procedures: 1, lying down for about two hours; 2, lying down for about one hour, then standing up without leaning against any object for one hour or more; 3, procedure 2 reversed. The subjects were not told to relax on lying down, but unless instructed to keep awake they frequently fell asleep. They were under observation while lying down, and it was thus possible to note the exact time at which they fell asleep and when they awoke. The pulse, respiratory rate and sometimes blood pressure were recorded in most cases. The time chosen for the tests was mid-afternoon, since that time coincides with the plateau in the temperature curve, or else shows only a minor spontaneous upward or downward trend. Tracings of typical curves are presented in figure 1, and all the results sum-

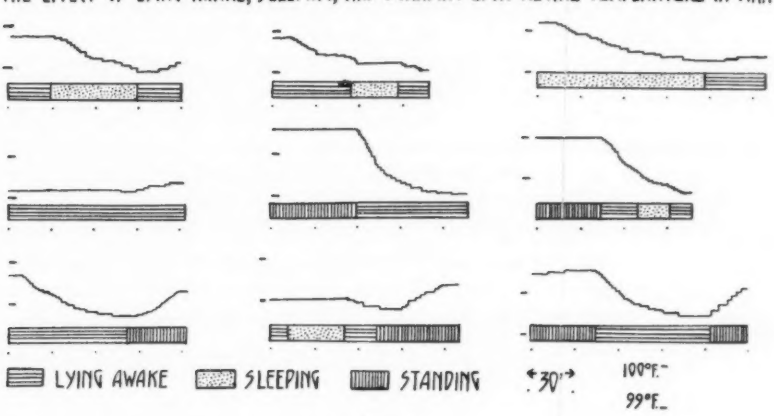

Fig. 1

marized in table 1. It will be seen that lying resulted in a fall in rectal temperature in the majority of tests, but in a considerable number of cases there is no change or even a slight rise. The tendency to a further fall in temperature becomes a little more accentuated with the onset of sleep, even though here too the temperature rose or remained stationary in some instances. For an average of sixty-nine minutes of lying down preceding sleep the average fall in temperature was 0.19° Fahrenheit, whereas for an average of fifty-two minutes of sleep the drop was 0.27° Fahrenheit. Upon awakening there is a very slight tendency to a further fall in temperature resulting in an average decrease of 0.03° Fahrenheit for a period of thirty-six minutes. On lying down, after standing up for about an hour, there was a fall in temperature in all tests made, and the fall was much more marked than on lying down without such a preliminary somewhat tiring

procedure. Likewise on standing up after lying down for an hour or more there was a rise in body temperature in every case.

The general trend of the body temperature on lying down, both before, during and after sleep is downward. The variability seen in individual temperature records is most probably due to the then present tendency for the body temperature to go up or down slightly. Even though the mid-afternoon hours represent a plateau in the temperature curve the actual peak may be closer to noon or nearer six o'clock in the same subject on

TABLE 1

The comparison between the rectal temperatures at the beginning and end of period of registration, in four subjects

PROCEDURE FOLLOWED	NUMBER OF CASES SHOWING AN INCREASE (+), NO CHANGE (=), OR DECREASE (−) IN TEMPERATURE					AVERAGE DURATION IN MINUTES (UPPER FIGURE) AND AVERAGE CHANGE IN TEMPERATURE IN DEGREES FAHRENHEIT (LOWER FIGURE) FOR ENTIRE SERIES OF TESTS				
	Subject I	Subject II	Subject III	Subject IV	All	Subject I	Subject II	Subject III	Subject IV	Average of all tests
Lying preceding sleep	4+		1+		5+	55	83	87	50	69
	11=	2=	1=		14=					
	3−	7−	9−	3−	22−	+0.03	−0.45	−0.21	−0.67	−0.19
Sleep	3+	1+			4+	57	57	26	53	52
	6=		1=		7=					
	16−	6−	3−	2−	27−	−0.24	−0.51	−0.13	−0.14	−0.27
Lying upon awakening	8+	2+			10+	34	40	17	55	36
	3=	1=	1=		5=					
	7−	6−	2−	2−	15−	0.00	−0.08	−0.07	−0.16	−0.03
Lying after standing (aver. 52')	7−	5−	4−	6−	22−	68	76	87	88	79
						−0.86	−0.82	−0.45	−1.30	−0.90
Standing after lying (aver. 76')	9+	7+	4+	4+	24+	56	45	55	55	55
						+0.30	+0.56	+0.36	+0.47	+0.41

different days. The actual change in temperature as recorded represents the algebraic sum of the downward tendency resulting from lying down and the spontaneous upward or downward trend of the diurnal temperature curve. That this is so is shown by the as yet unpublished records of rectal temperature on lying down late in the evening, when the temperature trend is definitely downward. In practically all cases the temperature fell under those conditions. This interpretation is also supported by the results obtained on lying down after standing up for some time. The fall in temperature under these conditions is so great that it always more than overbalances the slight upward tendency that may be present on certain days. This greater decrease in temperature is undoubtedly due to more

complete muscular relaxation resulting from the fatigue of standing. That the changed demands upon the circulatory system were not responsible for this difference is indicated by the observation that the decrease in heart rate and fall in blood pressure were no greater upon lying down after standing up than upon merely lying down. The rise in temperature upon standing after lying is probably due to the increased muscle tonus in that condition, and it was great enough to overcome any downward trend of the temperature curve that might be present at that time.

It may be concluded that a change in the position of the body from vertical to horizontal leads to a fall in temperature. If the supposition that this is due mainly, if not entirely, to muscular relaxation is correct, it would indicate that the diurnal temperature curve may be due to a diurnal change in the tonus of the skeletal musculature, and that the downward trend of the temperature curve in the evening represents a gradually increasing muscular relaxation. This would explain why, if one stays awake all night, somnolence becomes increasingly difficult to resist until about three or four o'clock in the morning, afterwards progressively decreasing in intensity. Under ordinary conditions, going to bed at night when the muscle tonus is low, leads to a further decrease in tonus (as indicated by the low temperature), thus producing an additional decrease in the number of proprioceptive impulses reaching the cerebral cortex and making continued wakefulness impossible. After the onset of sleep the temperature may or may not show a renewed drop, depending upon the degree of muscular relaxation already attained, and upon the time of the day or night. For the same reason awakening may result in a rise, no change, or a fall in temperature.

SUMMARY

1. Assumption of the horizontal position by man generally leads to a fall in body temperature.

2. The onset of sleep tends to accentuate the downward trend of the temperature preceding sleep.

3. Awakening is not characteristically followed by a change in body temperature.

4. Lying down after standing invariably leads to a marked fall in body temperature, and the opposite is true if this procedure is reversed.

We are greatly indebted to Mr. J. D. Zeller, Mr. J. Meshken, and Mr. S. Platt for their valuable help in obtaining the results reported.

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THE EFFECT OF INTRAVENOUS ADMINISTRATION OF LECITHIN AND SODIUM CHLORIDE ON BLOOD SUGAR VALUES

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In a previous investigation (Gregg, 1932) it was reported that intravenous infusion of lecithin emulsions either in physiological and hypotonic salt solution or of the saline solutions alone into dogs in many instances resulted in a rise of the R. Q. and of the heat production. The cause was not discussed, although the possibility naturally suggested itself that these changes might be attributable, in part at least, to greater carbohydrate mobilization and oxidation. If so, they should be associated with a detectable upward trend in the blood sugar values following such infusions.

PROCEDURE. To test this possibility, blood sugar values were determined in four dogs, two of them from the same series previously used for the determination of the dynamic action of lecithin. All the animals were of a quiet and gentle disposition and, as a result of training, would lie quietly on beds provided for them. They had all been rigidly maintained at a practically constant weight for four to six weeks prior to the experiments on a diet in the proportion of 75 grams of beef heart, 75 grams of cracker meal, 12 grams of lard and 5 grams of bone ash. Blood samples, taken 18 hours after food, from the saphenous or jugular vein were analyzed for "true" sugar by the method of Somogyi (1930). Samples were taken immediately before injection and at approximately 15 minute intervals thereafter for 2 to 3 hours. Control blood sugar curves were also obtained by drawing blood samples without previous injection. The solutions for intravenous administration (100 cc. of 0.70 and 0.90 per cent sodium chloride and 2, 3 and 5 per cent lecithin in physiological salt) were injected at body temperature. The conditions for injection were in all respects comparable to those previously reported. Thirty experiments were performed.

RESULTS. The composite results on all dogs and also typical curves for dog 7 are pesented in figure 1. It will be noted that of the salt solutions used only the hypotonic saline was effective in causing a measurable hyperglycemia. In both the average curve and in the indivdual curve a peak is reached some time during the first hour, after which it returns to

the base line in two to three hours. The maximum increase in blood sugar varies in different experiments, i.e., from 11 per cent to 20 per cent. Intravenous administration of lecithin emulsions in physiological salt gave no significant changes (8 of 11 experiments). The average maximum increase was 3.5 per cent. Control blood sugar curves exhibited no change from the basal values, the average maximum increase being 1 per cent.

A further point of interest in these experiments is the marked constancy of the basal blood sugar values obtained under these rather stringent condi-

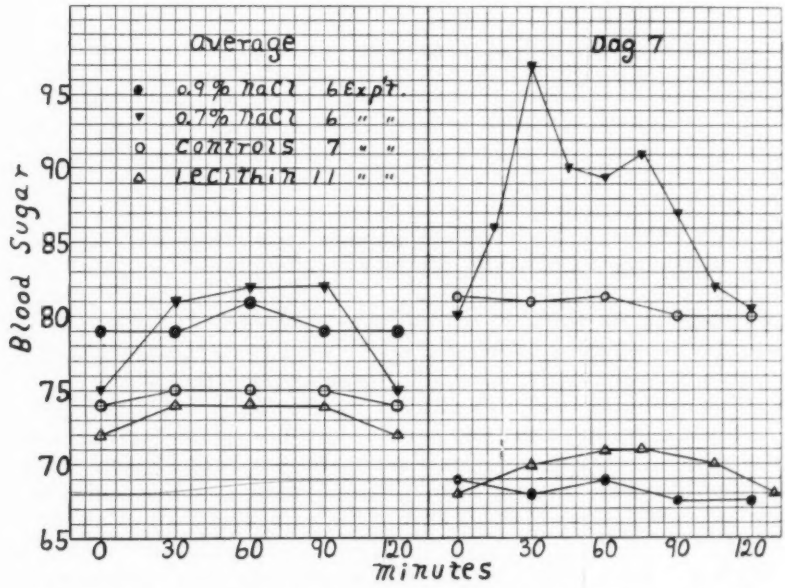

Fig. 1. "True blood sugar" values obtained after injecting 0.7 per cent, 0.9 per cent sodium chloride and 100 cc. of lecithin emulsions (3, 5 and 9 per cent lecithin) in physiological saline. Ordinates = milligrams per 100 cc. blood, abscissa = minutes after injection.

tions of diet, training and exercise. The four dogs gave the following average values: 74, 75, 71, 74 mgm. per 100 cc. blood.

CONCLUSION

Two facts stand out in these experiments: First, hypotonic saline is effective in raising the blood sugar, and secondly, lecithin emulsions given intravenously in physiological salt are non-effective. The conclusion naturally follows that the increase in the R. Q. and in the heat production (previously

reported) resulting from the injection of hypotonic salt or of lecithin suspended in 0.70 per cent salt may originate in part at least in the higher blood sugar values thus induced.

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THE EFFECT OF SHORT RADIO WAVES AND HEAT ON THE ELASTICITY OF THE AORTA

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Wiggers and Orias (1932) have recently reported circulatory changes following radiothermy which could not be explained fully on the basis of accepted hemodynamic principles. They might, however, be accounted for if the assumption were made that the elasticity of the aorta was altered. Experiments were therefore carried out to determine whether the passage of radio waves *per se* or by associated heating effects can cause alterations in the elasticity of aortic loops, removed immediately after death from dogs.

METHOD. Aortic segments exactly 1 cm. wide were cut by the method of Yater and Birkland (1930). Certain technical precautions emphasized by O. Frank (1928) were adopted. The loops of aorta were attached to a weight-lever system recording rectilinear ordinates on a drum and were immersed in a beaker of Tyrode solution which could either be heated by a flame or be placed between the plates of a G. E. radiotherm. Preliminary experiments showed that such passage of radio waves causes no detectable changes in the temperature of the solution or of parts of the apparatus connected to the loops.

RESULTS. The data obtained before and at various times during radiation were plotted as extensibility curves. Results such as are shown in figure 1 indicate definitely that the passage of radio waves *per se* does not alter the extensibility of aortic loops. Since the possibility of a selective heating in the body is not excluded, the effects of heating the solution directly were tested. Graphs such as those of figure 2 show conclusively that changes in temperature within physiological ranges (in this instance, 29.5–44°C.) do not alter the aortic extensibility. Only when excessive temperatures (60°–70°C.) are reached does the extensibility diminish definitely and in so doing follows Hooke's Law. After such a state is reached no great recovery occurs upon subsequent reduction of temperature to 30°C., indicating a permanent change in tissue.

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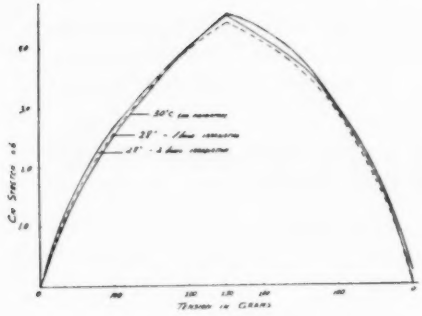

Fig. 1

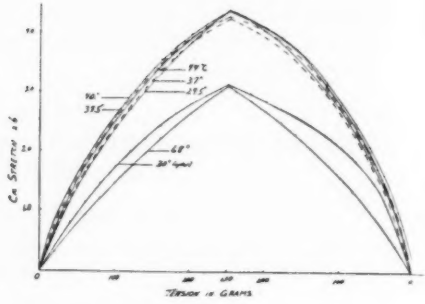

Fig. 2

CONCLUSIONS

The experiments give no evidence that the elasticity of aortic rings is affected either directly by passage of radio waves or by such elevation of temperature as is likely to occur in the body.

We wish to acknowledge the help of Dr. C. J. Wiggers, under whose supervision this work was carried out.

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THE EFFECT OF SHORT RADIO WAVES ON PERFUSED CATS' HEARTS

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Received for publication January 23, 1933

The bulk of experimental evidence indicates that the passage of high frequency electromagnetic vibrations (including short radio waves) has no specific effect on the functions of tissues and organs apart from those attributable to heating (cf. Christie and Loomis, 1929; Richard and Loomis, 1929; Fenn and Lachford, 1929; Nasset, 1932; Wiggers and Orias, 1932; Heinle and Phelps, 1932).

The experiments briefly presented in this paper supply further confirmatory evidence; short radio waves *per se* were not found to have any appreciable effect on perfused cats' hearts.

METHOD. Cats' hearts perfused with Tyrode solution at constant temperatures and pressures (Langendorff method) were mounted between the plates of a G. E. radiotherm supplying radio waves at a frequency of 10,000 K.C. Ventricular contractions were recorded by the suspension method and heart temperatures were read on a small thermometer inserted into the cavity of the right ventricle. After repeated trials an arrangement of the heart and supplying tubes was found by which heating of the perfusion fluid in the supplying cannula was obviated. Penetration of the heart by radio waves was tested by illumination of a small neon lamp, but was as a matter of fact assured by an increase in heart temperature of several degrees two minutes after the apparatus was turned on. By surrounding the inflow tubes with a water jacket in which the temperature could be quickly altered by water flow, the perfusion fluid could be cooled however to compensate for such elevation of heart temperature induced by radiothermy.

As a rule perfusion temperatures and pressures were kept around 30, for, in the experience of this laboratory, this seems to prolong the performance of perfused hearts.

RESULTS. Two types of experiments were performed. In one series, the progressive changes in rate and amplitude of ventricular contractions for one or two hour intervals were compared in irradiated and non-irradi-

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ated hearts. In numerous trials there was no indication that prolonged radiothermy had any effect on the natural decline in rate and amplitude, apart from a slight temporary increase, if the temperature was permitted

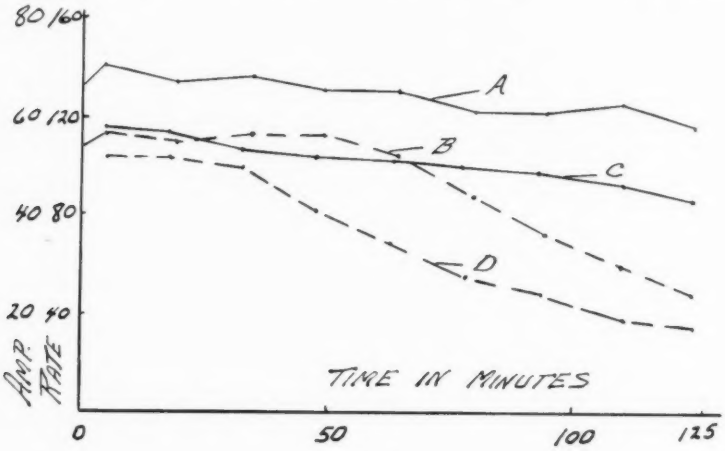

Fig. 1

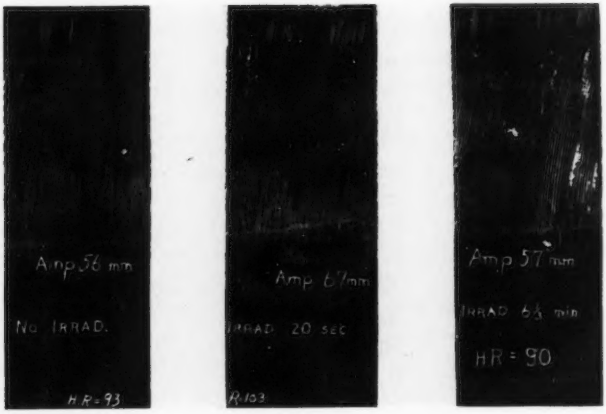

Fig. 2

to rise at the start. The effects are graphically shown by curves of figure 1 Curves *A* and *B* show changes in ventricular rate and amplitude respectively in a perfused heart submitted to radiothermy for a period of two hours. Curves *C* and *D* show similar sequential changes in an non-irradiated heart

perfused under conditions of constant pressure and temperature. The only alteration in the trend of the curves is the temporary increase in rate and amplitude coincident with the temporary initial increase in heart temperature from 33.8°C. to 35.8°C. In other experiments in which such a rise was compensated even this difference disappeared.

In another series of experiments, radiothermy was induced for shorter intervals, e.g., 1.5 to 12.5 minutes and the rise in temperature was compensated as quickly as possible. The curves of figure 2 show the nature of the results. The first segment represents a control record at 35°C. The second shows the increase in amplitude and rate as temperatures rose to 37°C. during 20 seconds of radiothermy. The third segment shows the return of normal amplitude and rate when temperatures were compensated and restored to the original level of 35°C.

Similar results were obtained in ten other experiments. Study of these data revealed sometimes a slight increase and sometimes a slight decrease in ventricular rates and amplitudes during the continuance of radiation for intervals of 1.5 to 14.5 minutes. However small the magnitude of the changes, the fact that they occur in different directions and are no greater than those found in non-irradiated hearts makes such small changes of no significance.

SUMMARY

Cats' hearts were perfused with Tyrode solution under constant temperature and pressure. Temperature changes produced in the heart were compensated by reducing the temperature of the perfusion fluid. It could not be shown in such preparations that radiothermy continued for 1 to 2 hours modified the natural tendency toward an increasingly hypodynamic state.

Short periods of radiothermy cause an almost immediate elevation of heart temperature which is accompanied by increased rate and amplitude of ventricular beats, but this disappears when the rise in temperature is compensated by reducing the temperature of the perfusion fluid.

We wish to acknowledge our appreciation to Dr. C. J. Wiggers for his guidance and supervision of this research.

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BONE MARROW VOLUME IN ADULT DOGS

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In a study of hemoglobin and red cell production in severe long continued anemia due to bleeding in the dog it is helpful to visualize the red marrow as a functional unit. A recent report (3) gives the histological picture and general ratio of red to fat bone marrow in various dogs in the anemia colony.

The functional capacity of the red marrow under these conditions is perhaps not fully appreciated. If we say that a dog of the type studied below has a red marrow volume of 300 cc., it can be stated with confidence (4) that this marrow could easily produce 120 grams of new hemoglobin or 300 cc. of packed red cells as the result of a diet intake of liver and iron lasting 2 weeks. If we are to push this analysis further we must say that in some dogs as much as half the total marrow volume may be fat and much of the red marrow consists of capillaries, stroma, a variety of white cells and mature red cells. Therefore it is safe to say that the parent red cells in the marrow can produce many times their own volume of mature red cells in an optimum diet period of 2 weeks. This is truly a respectable degree of productive activity and removes these red cells from the category of drones in the marrow hive.

It is difficult to visualize the red marrow in any mammal because of the fact that the marrow is so widely scattered throughout the skeleton. When the average physiologist tries to think of the bone marrow as a unit mass of marrow and fat cells he is at a loss for some object of comparable size and as a rule thinks in terms of the spleen. Most readers we believe visualize red marrow volume as equivalent to or less than the spleen volume. This is a serious error and one should think of the bone marrow as two-thirds the volume of the liver in the adult dog. From the few scattered reports in the literature dealing with marrow volume, one finds that the marrow amounts to 2.3 per cent of the body weight in infants (6) and 2.0 to 2.59 per cent of the body weight in adult rabbits (2). In the adult human the marrow percentage of body weight may be as high as 5 per cent (7), (1).

METHOD. The determination of marrow volume was carried out essen-

tially as described by Töppich (6) and Nye (2). Each skeleton was dissected free from soft tissues and disarticulated. Each skeleton was prepared at a separate time so that there could be no confusion of individual bones. The bones from each skeleton were placed in a container, covered with water and placed in an incubator at 37°C. The water was changed twice a day for six days during this process of maceration. The bones were then covered with Douglas broth, a 5 cc. culture of Clostridium histolyticum[1] added and incubation continued for six days at 37°C. in a closed vessel. Several litmus tests showed no acid present. The bones were then autoclaved, washed in running water by day and incubated at 37°C. at night for five days. Finally the bones were washed in running water continuously for 3 days. They were dried to constant weight at 47°C. and boiled in fresh ether under reduced pressure for ten minutes then allowed to remain in this ether for twenty-four hours. This was repeated six times using fresh ether each time. They were then carefully cleaned with a soft cloth to remove all foreign substances. They were dried at 100°C. to constant weight and the weight of each skeleton noted.

To facilitate ingress of agar into the marrow cavities small holes (1 mm. diam.) were drilled into the shafts of the long bones and ribs, bodies of vertebrae and flat bones of the pelvis. After agar impregnation under vacuum many bones were split to ascertain beyond any doubt that there had been complete filling of the marrow spaces.

The teeth and nails were discarded and not included in the skeletal weight. The skull presents some difficulties. The nasal sinuses including the large antra and smaller ethmoid spaces—also the large vascular and neural foramina all would introduce large errors due to retained agar. Moreover the red marrow represented in the base of the skull is small in amount. We decided to discard the base of the skull from our marrow material but included the calvarium and lower jaw. The entire skull was included in the bone weight.

The slight error introduced by this omission of some red marrow in the base of the skull is probably more than offset by errors inherent in this method which of necessity includes in the marrow volume all the nutrient foramina which obviously contain no marrow cells but only stroma and vessels.

The bones were carefully dropped into a boiling 4 per cent solution of agar agar and boiling was continued under reduced pressure for fifteen minutes. Each bone was picked from the solution, rapidly rinsed in hot water and plunged into iced water. They were carefully wiped with gauze to remove all excess agar. A large beaker was filled to overflowing with distilled water and the bones added one by one. The water displaced was caught and weighed in an evaporating dish.

[1] American Type Culture Collection Number 611.

To determine the specific gravity of dog bone several of these bones were boiled in water for a half-hour. They were put in fresh water and boiled another half-hour. This process was repeated four times to remove all agar, then they were dried in an incubator at 50°C. over night. Pieces from the ends and shafts of long bones and from vertebrae were broken in a mortar and sieved to remove the fine bone powder. The pieces used were about 1 mm. in thickness. A known sample was taken in a picnometer, covered with distilled water and boiled under reduced pressure for fifteen minutes to remove all air bubbles. The picnometer was then filled up with distilled water, carefully dried on the outside and weighed at 22°C. The following figures were obtained in separate determinations for the density of bone: 2.192; 2.185; 2.225; 2.174; 2.176. The average, 2.189, was used.

The volume of the bones is obtained by dividing the weight of the dry bones by the specific gravity. This volume subtracted from the volume of the bones filled with agar as found by the water displacement gives the volume of bone marrow. The results obtained in these calculations are found in table 3.

EXPERIMENTAL OBSERVATIONS. The two dogs used for marrow volume determinations were taken from the anemia colony in this laboratory. The dogs are bred and raised in the laboratory and their clinical history is complete from birth. The dominant strain is that of a short-haired white bull dog. The stock is not pedigreed and there is some coach dog and terrier blood in the colony. These two dogs were of the same litter, born September 17, 1930.

Dog 30-118 was a healthy female which showed some coach spots on a white coat. After weaning she was raised on a diet of salmon bread (8) and raw hamburger in equal amounts. The diet intake was gradually increased with growth of the pup up to a daily maximum of 200 grams bread and 200 grams meat. Cod liver oil, 20 cc., was added daily to the diet mixture. She was full grown at approximately 9 months and in all respects normal. This dog was of normal nutrition in contrast to dog 30-120 which was fat. When a year old the usual bleeding régime was begun to reduce the hemoglobin and red cells to the standard anemia level which is maintained between 40 and 50 per cent hemoglobin. Some blood volume data are given in table 1.

During the last 4 weeks the hemoglobin was allowed to return to normal and the dog was used for an x-ray experiment which caused death. Autopsy was done shortly after death (May 17, 1932) but the findings do not concern these marrow volume studies. The skeleton was used as described above.

Dog 30-120 was a healthy female which showed an occasional grey spot on a white coat. As in the case of the other dog she was healthy through-

out her life. After weaning she was put on a diet of salmon bread, canned salmon, "Klim" with added cod liver oil. The ration was increased gradually with growth of the pup up to a daily maximum of 300 grams salmon bread, 150 grams canned salmon, 40 grams Klim and 20 cc. cod liver oil. She was full grown at 9 months and decidedly fat, weighing 4 kilos more than her litter mate 30–118. She was made anemic at the same time as the other dog and some blood volume data are given in table 2.

TABLE 1

Anemia history

Dog 30-118

	SEPTEMBER 3, 1931	OCTOBER 8	DECEMBER 10	JANUARY 7, 1932	MARCH 30
Weight, kgm.	13.4	13.35	13.8	13.9	12.62
Blood volume, cc.	1,081	1,054	830	1,125	1,048
Plasma volume, cc.	531	797	657	855	809
Blood per kgm., cc.	81	79	60	81	83
Hb, per cent	134	52	41	47	44
RBC, per cent	50.5	23.8	20.3	23.6	22.4
RBC, MIL.	6.430	4.220	4.110		5.700
Color index	1.05	0.62	0.50		0.40
Hb. index	2.66	2.19	2.04	2.00	1.97

TABLE 2

Anemia history

Dog 30-120

	SEPTEMBER 3, 1931	OCTOBER 8	DECEMBER 10	JANUARY 7, 1932
Weight, kgm.	16.2	16.35	16.0	16.5
Blood volume, cc.	1,223	1,079	1,178	1,211
Plasma volume, cc.	536	808	889	916
Blood per kgm., cc.	76	67	74	74
Hb, per cent	159	59	51	53
RBC, per cent	55.8	25.3	23.6	23.8
RBC, MIL.	8.330	4.930	4.830	
Color index	0.96	0.60	0.53	
Hb. index	2.86	2.35	2.14	2.22

The anemia was continued until February 12, 1932, when bleeding was discontinued and the blood hemoglobin promptly came back to normal. Dog was given ether (March 4, 1932) and perfused by plasmapheresis to free the tissues from contained blood. Autopsy was done at once and the viscera were all normal and edema was not present. The skeleton was used as described above.

A glance at table 3 shows that these two litter mates had quite similar volumes of marrow—289 cc. and 314 cc. respectively in spite of a weight difference of 4 kilos. This weight difference was largely fat and we note that the liver weights are identical. The weight of the bone in the skeleton is 539 and 514 grams respectively or using the bone specific gravity of 2.189 we record the bone volume as 246 cc. and 234 cc. The weight of the marrow plus bone is equivalent to the true skeletal weight or 828 grams and 828 grams respectively. We accept the marrow specific gravity as one (Mechanik).

Blood and plasma volume figures and method difficulties have been discussed fully elsewhere (5). The plasma volumes increase with the bleeding to produce anemia—this is a compensatory phenomenon which is uniformly observed (tables 1 and 2). The general normal plasma volumes are at the lower limits of normal.

TABLE 3

Volume of marrow, liver, spleen and blood

	DOG 30-120		DOG 30-118	
	Total weight	Percentage of body weight	Total weight	Percentage of body weight
	grams		*grams*	
Body	16,318		12,181	
Marrow	314	1.9	289	2.4
Bone	514	3.1	539	4.4
Bone + marrow	828	5.1	828	6.8
Liver	470	2.9	470	3.8
Spleen	40	0.24	25	0.20
Blood volume	1,220	7.5	1,080	8.8
Plasma volume	536	3.3	531	4.3

DISCUSSION. It is obvious that the red marrow, spleen and liver are intimately related in the production, maintenance and disposal of red cells and hemoglobin. There is evidence that the liver is essential for the production of the parent substances which go to form the mature red cells and hemoglobin in the marrow. For this reason it is helpful to keep in mind the volumes or weights of these various organs together with the circulating blood and plasma volumes.

We may give the following illustration. A dog of the character described above of average nutrition weighing 15 kilos should have a blood volume of 8 to 9 per cent (1200–1350 cc.); a plasma volume of 4 to 5 per cent (600–750 cc.); a liver weight of 3 to 3.7 per cent (450–550 gm.); a bone marrow weight of 2 to 2.5 per cent (300–450 gm.); and a spleen weight of 0.2 to 0.5 per cent (30–75 gm.).

In four other animals from the anemia colony the body weights were 11988–17613–14829 and 13153. The liver weights were 380 (3.1 per cent)—470 (2.6 per cent)—520 (3.5 per cent) and 495 (3.7 per cent). The spleen weights were 23 (0.19 per cent)—80 (0.45 per cent)—68 (0.45 per cent and 40 (0.3 per cent). The average percentage of body weight for the liver = 3.25 and for the spleen = 0.31. In this average we include the two dogs given in table 3.

The bone marrow volume is relatively constant in a young adult dog but the ratio of red to fat marrow is a variable which is under constant adjustment depending on the need for red cells and many other factors. In the growing animal the red marrow may completely fill all marrow spaces and eliminate all fat cells. This is true in certain cases of experimental anemia (3). In old age the red marrow shrinks, the marrow spaces expand due to osteoporosis and the fat cells fill up the surplus area.

SUMMARY

Two young adult dogs (litter mates) in the anemia colony showed marrow volumes of 289 cc. (2.4 per cent body weight) and 314 cc. (1.9 per cent), the low percentage 1.9 per cent being due to increased body weight (fat).

The marrow volumes or weights in these two dogs are about two-thirds of the liver weights (61 and 67 per cent).

It is obvious that marrow volume will be relatively higher in animals in poor nutrition and still higher when age introduces osteoporosis.

It may be that a more stable ratio for comparison will be the liver-marrow ratio as body nutrition has relatively slight influence on the liver and bone marrow volume.

The ratio of red to fat marrow is constantly fluctuating due to age, anemia and other factors related to production, maintenance and conservation of hemoglobin. This ratio may vary from 100 per cent red marrow with fat excluded to 20 per cent red and 80 per cent fat marrow, both extremes observed in the anemic dog.

The parent red cells in the red marrow under optimum conditions in a 2 week period may produce many times their own volume of mature red cells.

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EFFECT OF RESPIRATION ON CARDIAC OUTPUT

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The influence of negative pressure in the thorax and the alteration of this pressure associated with the different phases of respiration are generally considered to be of considerable importance in facilitating the return of blood to the right heart. Numerous studies have been made of pressure variations in the right heart and pulmonary circuit with variations in lung expansion brought about by artificial ventilation with open thorax, but observations with intact thorax and normal breathing are few. The literature up to 1921 has been critically reviewed by Wiggers (1), and references in the present report will be confined to more recent reports and to those of especial significance in connection with the phase of the subject under discussion.

So far as we have been able to find, measurements of heart output as related to the different phases of respiration have been carried out only with open thorax, with the single exception of the use of an indirect method by Anderez and Cloetta (2); a method which has been shown to be invalid by subsequent workers. It has been generally assumed that increased rate and depth of respiration increases the "aspiratory" effect of the thorax and this, by increasing inflow of blood, is a factor in the increased heart output accompanying muscular exertion (3), but there appears to be no direct experimental evidence in support of this view.

METHODS. Dogs were used under ether anesthesia. With intratracheal artificial respiration by means of an interrupted air blast, a rib was resected for part of its length and a plethysmograph enclosing the two ventricles was applied. Several types of plethysmograph, including metal and molded wax models were tried, but entirely successful results were obtained only with a double wall cardiometer similar to the type described by Henderson (4). The outside wall was of stiff rubber, the inner of thin dental rubber, the two walls being cemented together at the opening applied around the auriculo-ventricular groove. In order to prevent encroachment upon neighboring vessels and to avoid any undue reduction in the total thoracic volume, the smallest size adaptable to the heart was used. The space between the two walls was connected by a large tube (8 mm. internal diameter) to a metal piston recorder. The piston was

carefully ground to fit, and its weight counterbalanced. The thorax was closed; the lungs before the final closure being distended by a continuous air flow to completely fill the thorax. The tracheal tube was removed and normal respiration reëstablished.

Since the air space in the plethysmograph was sealed off from the thoracic cavity, there was no possibility of air leakage from the piston recorder or

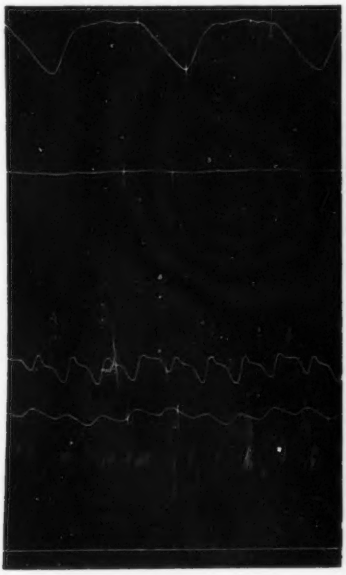

Fig. 1

Fig. 2

Fig. 1. Intrathoracic pressure, auricular venous pressure recorded by water manometer, ventricular stroke volume, arterial pressure (femoral) and base line for arterial pressure, in sequence from top to bottom of record, approximately one-third size of original record. The speed of record before reduction, 42 mm. per second. Taken during normal quiet breathing under ether anesthesia. Four millimeters of change in respiratory record correspond to 1 mm. of water pressure.

Fig. 2. Similar to figure 1 and with same reduction. In another animal after section of both vagi. Speed of record before reduction 5.2 mm. per second.

connecting tube into the cavity. The only possible leakage was into and out of the thin bag surrounding the heart through the contact at the auriculo-ventircular groove. The accurate position and fit of the plethysmograph was confirmed in each case after death and the possibility of a leak was further tested by forcibly distending and contracting the lungs by pressure and suction after the heart had stopped beating and before the chest was opened. The changes in intrathoracic pressure thus produced

far exceeded those present during life, and only those experiments were included in which this test was successful, that is, when extreme changes in intrathoracic pressure were without demonstrable effect on the piston recorder.

Arterial pressure was recorded from the femoral artery by means of a mercury manometer. Pressure in the right auricle was recorded by means of a tube passed into the auricle through the right external jugular vein and connected with a water manometer. Intrathoracic pressure was recorded by a large trocar introduced into the thorax connected with a calibrated tambour.

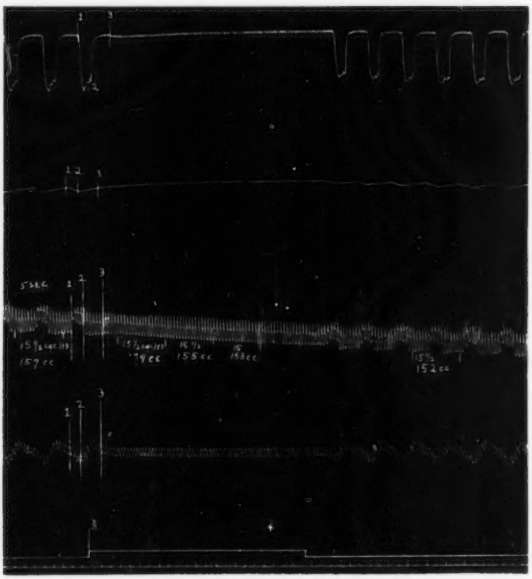

Fig. 3. Similar to preceding figures. Stimulation of central end of vagus (marked by base line pen) with weak current. Both vagi cut. Time in second intervals.

Results. In all observations there was a consistent relation between inspiration, auricular pressure and stroke volume. During the fall in intrathoracic pressure there was a diminished stroke volume and an initial small rise, succeeded by a fall, in auricular pressure. The extent of the changes in stroke volume and auricular pressure were related to the depth of breathing and hence to the extent of fall of intrathoracic pressure during inspiration. The fall in auricular pressure was always less than, usually about one-fourth, the fall in intrathoracic pressure. In figure 1, the first stroke volume during the inspiratory phase was reduced 12.5 per cent and

the second 50 per cent from that present during the expiratory pause. The changes in auricular pressure were less than 1 mm. of water, while the intrathoracic pressure was reduced approximately 5 mm. of water. In figure 2, taken during the very deep respirations following section of both vagi, the changes in auricular pressure and in stroke volume are exaggerated. It is to be noted that in this and in similar records where the respiration is excessive that the decrease in stroke volume is associated with an increase in the diastolic volume of the ventricles.

Figure 3 is an example of arrest of breathing by weak stimulation of the central end of the cut vagus nerve. There is a slight and transitory de-

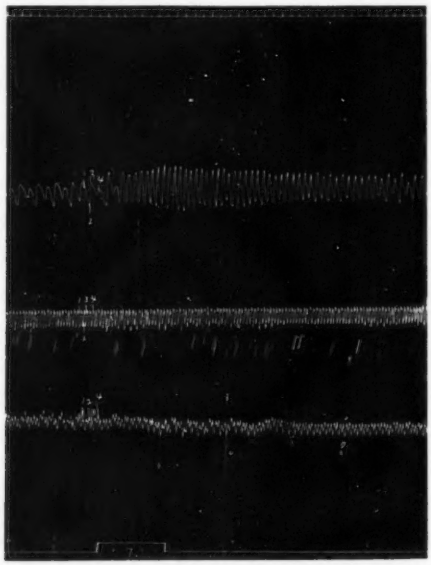

Fig. 4. Similar to preceding records except that auricular pressure is not recorded. Effect of stimulation of central end of saphenous nerve. Vagi intact.

crease in stroke and minute volume during the first part of the apnea, but these later return to an approximate normal before the apnea is terminated. Auricular pressure shows a gradual but slight rise (3 mm. of water) through the apneic period. Figure 4 shows likewise the relatively small effect upon stroke volume and minute output during a period of marked hyperpnea, resulting from weak stimulation of the central end of the saphenous nerve.

Changes in systemic arterial pressure were inconstant. In normal quiet breathing the relation usually described in the dog, a rise during inspiration was observed, while in the deep breathing after vagus section the opposite effect was almost always obtained.

DISCUSSION. A rather unexpected result was the decrease in stroke volume during inspiration. It should be noted that the method used gives the sum of the stroke volumes of the two ventricles and does not differentiate between the two. This diminished total stroke volume was invariably associated with a fall of auricular pressure. This fall is not as great, however, as the associated inspiratory fall of intrathoracic pressure, hence it is evident that the effective auricular pressure (auricular pressure minus intrathoracic pressure) is increased. One would expect from this an increased filling of the right ventricle and presumably an increased stroke volume of this chamber. At the same time, however, the expansion of the lungs results in a larger vascular capacity in the pulmonary circuit (5, 6), and a fall of pulmonary arterial pressure (7). This would in turn reduce the flow into the left auricle and ventricle with a lessening of the stroke volume of this chamber and the net result might well be a diminished total discharge from the two chambers. It has been shown that the response of the left ventricle to changes in filling pressure is greater than the right (8). When the changes in intrathoracic pressure are exaggerated after section of the vagi, the reduction in total ventricular stroke is also more pronounced, while the diastolic filling is usually greater than normal (figs. 2 and 3). This may be explained by the increase in effective filling pressure upon the thin walled right ventricle. The diminished output from the left ventricle is also indicated by the sharp fall in arterial pressure that occurs at this time (figs. 2 and 3).

Another factor tending to diminish stroke volume during inspiration when the vagi are intact is the usual acceleration of the heart which occurs at that time.

The fact that extreme alteration of respiratory activity from a condition of apnea lasting for a half minute or more (fig. 3) to that of marked hyperpnea (fig. 4) does not, under the conditions of our experiments, alter to any marked degree stroke or minute volume, would suggest that the importance of breathing in reference to venous return has been exaggerated in the usual theoretical discussions of this subject. It would appear that the more important factor is the average pressure gradient on entering the thorax, rather than the alteration of this gradient ("aspiratory action") associated with variations of respiratory activity.

The variable effect of the phase of respiration on systemic arterial pressure may depend upon the relation between heart rate and respiratory rate. It has been found that at least three heart beats intervene between the instant of marked reduction of venous inflow into the right heart and the resultant fall in systemic arterial pressure (9).

It should finally be noted that the main difference in our experiments from the normal condition in the intact animal was the fact that the ventricles were exposed, not to intrathoracic but to atmospheric pressure.

While it is usually stated that the changes in intrathoracic pressure probably affect the thick walled ventricles little, this may not be true in their relaxed state and under the low internal pressure present in diastole. This factor is a continuous one, however, and does not appear to us to enter into serious consideration in the comparative results obtained.

CONCLUSIONS

1. In dogs under anesthesia in which stroke volume and minute cardiac output from the two ventricles is measured by a cardiometer during normal breathing, there occurs during inspiration a diminished stroke volume and a slight fall of right auricular pressure. The effective venous pressure is increased, since the fall in auricular pressure is not as great as the fall in intrathoracic pressure. The diminished total stroke volume may be explained by diminished left ventricular output due to retention of blood in the increased pulmonary bed.

2. In the greater reduction of intrathoracic pressure associated with the deep inspirations following vagus section, these effects are exaggerated and in addition the diastolic volume is increased.

3. Under the conditions of these experiments, marked alterations of breathing have little effect on the average stroke volume or on minute volume when considered over a period of time. It is believed that the influence of the extent of breathing on venous return is not as great as is ordinarily stated.

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OBSERVATIONS ON THE BLOOD OF NORMAL HUMAN SUBJECTS DURING FETAL LIVER FEEDING

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The erythropoietic rôle of the fetal liver indicates it as a substance not to be overlooked in the research for a substance to stimulate hemoglobin synthesis. Dried fetal calf liver was accordingly introduced by Berglund, Watkins and Johnson (1928a, b, c) in the treatment of several types of anemia. These investigators reported an accelerated regeneration of hemoglobin following fetal liver. Upham and Nelson (1930) report favorably upon the use of fetal calf liver in a case of primary aplastic anemia. Sherwood (1931) has employed fetal liver in three cases with beneficial effect.

That normal individuals respond to liver extract feeding with an immediate and striking increase in erythrocytes was reported by Watkins, Johnson and Berglund (1928). During a ten-day feeding period, they observed a pronounced rise in the cell count of six subjects, as great an increase as 140 per cent of control value being recorded. Acrocyanosis, epistaxis, headaches and other symptoms of polycythemia were recorded. Crane, Howard and Murphy (1930) in a similar experiment found only a moderate rise in red blood cells during a fourteen-day liver extract feeding period.

The present study was undertaken to observe similarly the effect of fetal calf liver on the blood of normal individuals. It was hoped thus to demonstrate the salient differences, if any, between fetal calf liver and adult beef liver, the one having been observed to accelerate hemoglobin synthesis and the other to stimulate unusual cell increase. Some subjects were given desiccated stomach, and for purposes of comparison with preceding investigations, others were fed liver extract.

PROCEDURE. Twenty university students between the ages of twenty-one and thirty-one were used as subjects. Of these, eighteen were men, apparently in perfect health, and two were women, both slightly anemic, but otherwise also in good health. Observations were made during and following two different feeding periods, each preceded by a control period of three to seven days.

The first feeding period was ten days in length. During this time,

five subjects received fifty grams powdered fetal liver daily, the equivalent of 300 grams of fresh fetal liver; two subjects received three vials of liver extract (N. N. R. no. 343)[1] daily, the equivalent of 300 grams fresh beef liver; three subjects received twenty grams desiccated stomach (ventriculin) daily. The observations were continued for twelve days following the close of this feeding period.

The second feeding period employed ten additional subjects. Of these, four ingested a daily dose of fifty grams powdered fetal liver for forty-five days. One subject discontinued the liver early in the series. Five individuals ingested daily fifty grams chocolate malted milk in a glass of cow's milk, paralleling the fetal liver subjects during the first eighteen days of the second feeding period.

The powdered fetal calf liver, liver extract and desiccated stomach were fed after being mixed with fifty grams sweetened chocolate malted milk in a glass of cow's milk to render palatable. No attempt was made to alter otherwise the diet or to regulate the physical activities of the subjects in any way.

Observations on the blood were made every other day. Blood for counting and determinations was drawn from an arm vein between 9:30 and 10:30 in the morning. A 10 cc. syringe was used and six cubic centimeters of blood were drawn. Of this, 5 cc. were placed in a 15 cc. graduated centrifuge tube containing exactly 1 cc. of 1.6 per cent sodium oxalate solution. Blood for counting was diluted with Hayem's solution and the pipettes were shaken for 3 minutes in a mechanical shaker. Two dilutions were made from each sample of blood and a count was made from each pipette. The count for each day was recorded as an average of the two pipettes. Reticulocytes were counted by the method of Friedlander and Wiedemer. Hemoglobin was determined by the Newcomer method, readings being made by north daylight illumination after the acid hematin solution had stood one hour. Hematocrit values were obtained by centrifuging the oxalated blood one-half hour at 2500 r.p.m. All enumerations, readings and manipulations during the investigation were made by the same individual.

Data from representative subjects are given in figures 1 and 2. The mean erythrocyte count for each subject's series was calculated, together with the deviation of each count from the mean. Similar calculations were made for the hemoglobin values. The probable error and the ratio of deviation from mean to the probable error were also found by use of the usual formulae.

Results. 1. Statistically significant variations occurred not only during and following feeding periods, but also in control periods and in control subjects. These significant variations are seen both above and below

[1] Furnished by Eli Lilly & Co.

the usual limits of statistical importance (fig. 1). Only moderate rises were noted in the erythrocyte and hemoglobin levels during or following feeding periods, no enumerations over 6.44 millions being found. No periodicity is noticeable, although sudden rises and falls occurred frequently in all subjects.

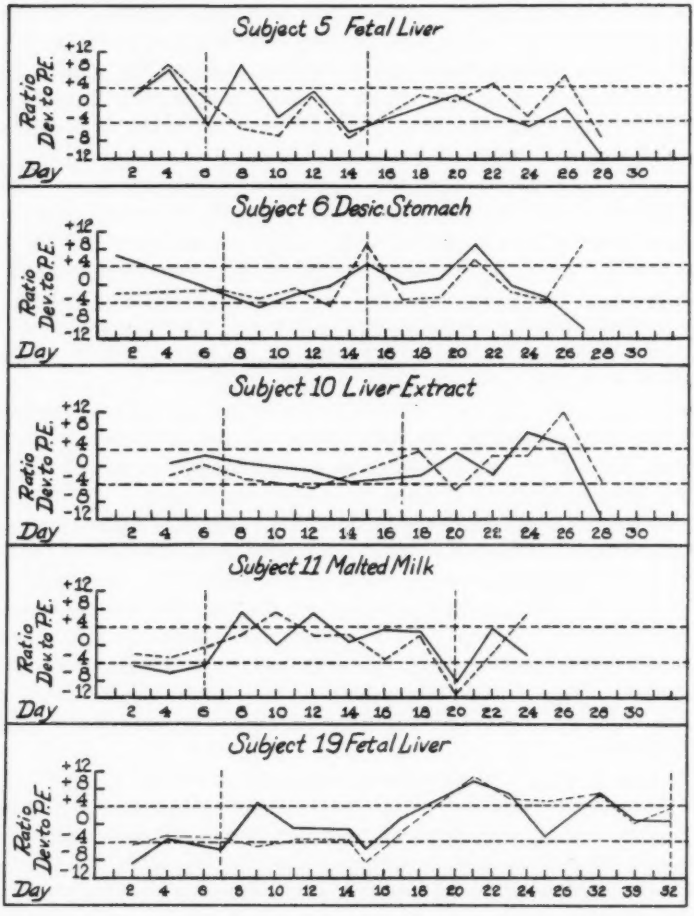

Fig. 1. Diagram to indicate statistical significance of erythrocyte (solid line curve) and hemoglobin (broken line curve) observations for five typical subjects. The ordinate represents the ratio of deviation of each observation from the mean to the probable error. Fluctuations between the horizontal broken lines are not considered statistically significant. The vertical broken lines indicate the beginning and close of the feeding period.

2. Withdrawal of fetal liver after a short feeding period resulted in all five cases in a decided fall of the erythrocyte level, after a latent period of several days, to a figure below the normal control level (subject 5, fig. 1). The drop did not appear in any of the four cases in which fetal liver feeding was continued for 45 days, as long as the substance was being fed (subject

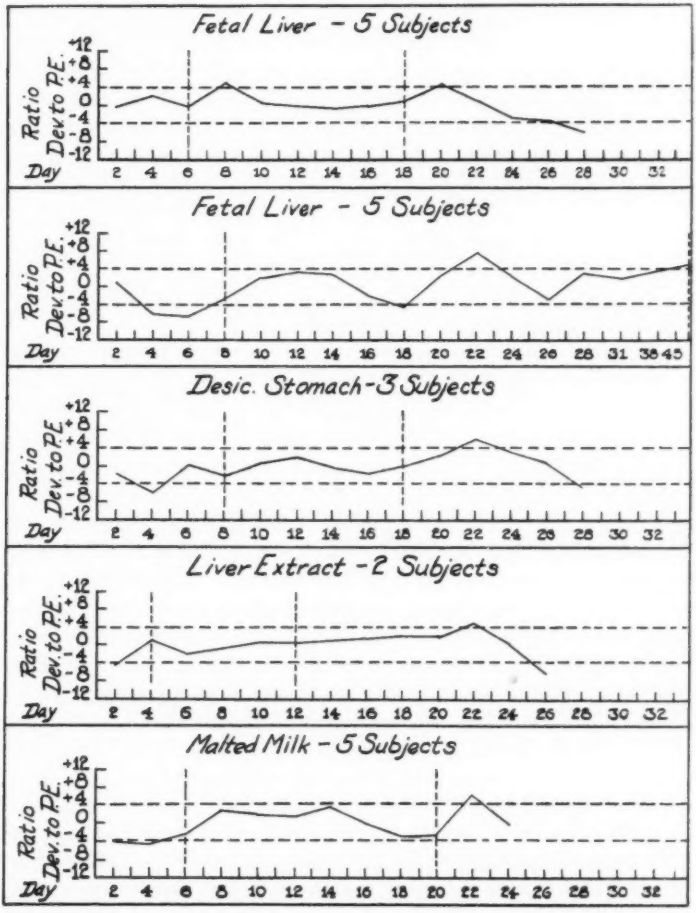
Fetal Liver - 5 Subjects
Ratio Dev. to P.E.
+12
+8
+4
0
-4
-8
-12
Day
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 31
Fetal Liver - 5 Subjects
Ratio Dev. to P.E.
Day
2 4 6 8 10 12 14 16 18 20 22 24 26 28 31 38 45
Desic. Stomach - 3 Subjects
Ratio Dev. to P.E.
Day
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32
Liver Extract - 2 Subjects
Ratio Dev. to P.E.
Day
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32
Malted Milk - 5 Subjects
Ratio Dev. to P.E.
Day
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32

Fig. 2. Diagram with composite curve for all subjects in each group, showing statistical significance of erythrocyte counts. The ordinate is an average of the various individual ratios of deviation of each observation from the mean to the probable error. Fluctuations between the horizontal broken lines are not considered statistically significant. The vertical broken lines indicate the beginning and close of the feeding periods.

19, fig. 1). This fall in red cell level below the control figure was also noticed in two of three subjects after taking desiccated stomach (subject 6, fig. 1) and in one of two subjects following liver extract feeding (subject 10, fig. 1). The fall in cell count which succeeds the withdrawal of liver or stomach substance is clearly seen in the composite curves (fig. 2).

3. No increase above normal was noted in reticulocytes. Counts were made for ten of the twenty subjects.

4. One normal subject, accustomed to occasional nosebleed, reported an instance of epistaxis during a period of fetal calf liver feeding, but none of the other symptoms recorded by Berglund were observed in any subjects.

5. Subject 19, a Negro girl, had been experiencing intermittent nosebleed for some time preceding observation, but the bleedings had stopped when she volunteered as a subject. A careful study of her case gave negative findings in physical examination, x-ray examination of the chest, and in urine and Wassermann tests. The white cell count was 7,800. Under fetal liver feeding there was a slight rise in hemoglobin and cell count which was sustained for the duration of the experiment (subject 19, fig. 1), a total of 52 days for this subject.

The diagrams showing ratio of deviation from the mean to probable error demonstrate clearly that statistically significant variations occur not only during and following feeding periods, but during control periods and in the control individuals receiving only malted milk. The statistical importance of readings in a given series being demonstrated, the task remains of establishing any given stimulus as the cause of the significant variations. Such a relationship would be indicated only if the response followed the stimulus similarly and consistently in a sufficient number of cases.

In assigning a cause to any significant fluctuation in red blood cells, the following must be considered: *a*, diurnal changes; *b*, increases due to exercise; *c*, unexplained day-to-day variations, and *d*, emotional rises. It is likely that other factors such as alterations in plasma volume may cause changes great enough to be of statistical significance. Leake, Kohl and Stebbins (1927) found diurnal variations in erythrocyte count averaging as high as 345,000 in six normal men. Hawk (1904) in making observations upon athletes, observed that the red cell count might show increases in exercise varying from 400,000 to 1,460,000 in a brief period of time. Smith (1932) found that such increases following very short periods of exercise were sustained for about 45 minutes. Smith (1931) also noted that the red cell count may vary from day to day in statistically significant amounts without observed cause, although she found no significant diurnal variation. Cannon (1929) states that the erythrocyte count increases in the cat 20 to 50 per cent during emotional disturbances.

With statistically significant variations, both high and low, occurring during all periods of both test and control subjects, it is virtually impossible

to fix any response as being definitely the result of liver or stomach tissue feeding, due to the difficulty of ruling out the variations named above. Further investigation of these variations has emphasized the effect of rest in lowering erythrocyte count.

One change is striking in its consistency. Withdrawal of fetal calf liver resulted in all five cases observed in a fall of erythrocyte level to a figure below the normal control value. Withdrawal of desiccated stomach showed a drop in one of three subjects, and discontinuation of liver extract showed a pronounced fall in one of the two subjects. In nearly every case, the fall occurs twelve or thirteen days after the feeding has been discontinued. Such a drop in red cell level was not observed during the 45-day fetal liver feeding period.

This fall was noted by Berglund and his associates after liver extract feeding. They suggested that it might represent a compensatory mechanism on the part of the body, operating to maintain a constant level of red blood cells. Since Crane, Howard and Murphy (1930) found no indications of increased cell destruction, it may be suggested that perhaps an endogenous factor which stimulates the normal maturation and liberation of erythrocytes ceases to be formed by the body when the same factor is furnished from an exogenous source.

McHargue (1925) has shown that the fresh substance of fetal (five-day premature) calf liver contains approximately thirteen times the amount of copper present in the fresh liver of a mature ox. Our analysis of the fetal calf liver used in these studies shows a copper content of twenty times the amount observed by McHargue in adult beef liver. The liver powder used by us was prepared from fetuses less than 70 centimeters in length. The comparative analyses are as follows:

	MGM. COPPER PER KGM. FRESH MATTER
Mature ox liver (McHargue)	12 5
Fetal calf liver (McHargue) (5-day premature)	161 3
Fetal calf liver (this study) (fetuses under 70 cm.)	250 0

Morrison and Nash (1930) observed in the livers of infants six times the average amount of copper present in adult livers. They also noted a very low copper content associated with severe anemia. These observations suggest that fetal calf liver therapy might be particularly beneficial in the type of anemia which seems to be associated with copper deficiency. The pronounced disparity in copper content is the most striking and perhaps the most significant difference between adult beef liver and the calf liver prepared from young fetuses.

SUMMARY

1. Observations made on eighteen normal and two slightly anemic subjects indicate that variations statistically significant, both high and low, occur during control periods and in control subjects, as well as during and following the feeding of fetal liver, desiccated stomach and liver extract. Some factors which may cause such fluctuations are considered.

2. Withdrawal of fetal liver resulted in a fall of erythrocytes below control value after a latent period in the five cases observed. A similar tendency was noted following stomach and liver extract feeding.

3. One anemic subject, after undergoing liver therapy for 45 days showed a slight but sustained rise in erythrocytes and hemoglobin.

4. A difference between fetal calf liver and adult beef liver of possible significance and value is the unusually high copper content of the former.

The authors acknowledge their indebtedness to Prof. Henry Werner of the University Foods Laboratory for furnishing an analysis of the fetal liver used in the foregoing experiments.

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THE GASTRIC HUNGER MECHANISM

IV. The Influence of Experimental Alterations in Blood Sugar Concentration on the Gastric Hunger Contractions

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There is much evidence that carbohydrate metabolism is in some way correlated to the motility of the stomach. Luckhardt and Carlson (1914) found that, during starvation, the motility of the stomach is augmented. Under certain dietary conditions, insulin hypoglucemia is accompanied by an increase in the gastric hunger contractions: Bulatao and Carlson (1924), for the dog, and Simici, Guirea and Dimitriu (1927), for man; Koref and Mautner (1926), for man, Mulinos (1927), for dog, and Quigley, Johnson and Hallaran (1929), for dog and man. Bulatao and Carlson (1924) reported that the stomach could be depressed by the intravenous administration of strong glucose solutions. This was denied by Mulinos (1927), whose findings were later confirmed by Quigley and Hallaran (1932). Pancreatectomy diabetes in dogs (Carlson, 1919), and pathological diabetes in the human are also accompanied by gastric hypermotility.

In the present paper are reported details of experiments which throw light upon the mechanism involved in the production of gastric hypermotility in diabetes. The concept is defended, that these conditions of pancreatic deficiency and of altered carbohydrate metabolism are related only incidentally to the increased gastric motility which accompanies them.

Experimental. The experiments were performed on female dogs, with gastric fistulae. The animals were trained to lie quietly for hours, while the gastric motility was graphically recorded by means of the balloon method of Carlson. Blood sugar determinations were made after the method of Schaeffer and Hartmann (1920).

Results. The experiments may be conveniently divided into four classes, as follows: *a*, gastric motility and spontaneous variations in blood sugar concentration; *b*, gastric motility as affected by intravenous injections of glucose solution; *c*, gastric motility under insulin hypoglucemia; and *d*, gastric motility in pancreatectomy diabetes.

a. Carbohydrate and hemoglobin concentrations were determined in blood drawn during various types of gastric hunger activity. The following protocol is typical for the entire series.

Dog 1. Weight 25 kilos

DATE	TIME	BLOOD SUGAR	HEMOGLOBIN	GASTRIC ACTIVITY
		per cent	*per cent*	
3-29-26	9:00	0.095	100	Tetanus
	9:45	0.072		Quiescent
	10:50	0.074	101	Quiescent
3-30-26	8:40	0.070	100	Strong type II
	9:38	0.063	102	Quiescent
	11:00	0.068	99	Quiescent

There was no correlation between the type of activity recorded and the blood sugar concentration.

b. In this group of experiments, the blood sugar level was raised artificially by means of 50 per cent glucose solution injected intravenously. The dose was 1 to 2 cc. per kilogram of body weight. The smallest volume of fluid injected into any animal was 10 cc., whilst the largest was 30 cc. The injections were made rapidly, in order to raise the blood sugar to as high a level as possible. No signs of any circulatory or respiratory disturbance were observed either during or immediately after the injections.

In a little less than 10 per cent of 87 injections into 11 different dogs, over a period of 30 months, the injection was followed by gastric depression. The periods of depression lasted from 5 to 30 minutes. Invariably within forty-five minutes after the injection, the gastric motility had returned to its previous level. Yet at this time the sugar of the blood was considerably higher than before the injection.

Dog 2. Female. Weight 18 kilos

DATE	BLOOD SUGAR	HEMOGLOBIN	GASTRIC ACTIVITY	COMMENT
	per cent	*per cent*		
(1) 5-10-26	0.097	100	Tetanus	Before injection
	0.305	89	Quiet	20 minutes after injection of glucose, 27 cc., 50 per cent I. V.
	0.208	94	Tetanus	10 minutes later
(2) 5-14-26	0.099	100	Tetanus	Before injection
	0.202	92	Tetanus	30 minutes after injection of glucose, 18 cc., 50 per cent I. V
	0.112	101	Type I	1½ hours later
(3) 6- 8-26	0.101	100	Tetanus	Before injection
	0.179	98	Quiet	12 minutes after injection of glucose, 12 cc., 50 per cent I. V.

In experiment 1 the stomach activity returned while the blood sugar level was well above normal. In experiment 2 the gastric tetanus was not affected by the injection, despite the fact that the blood sugar concentration had risen to 202 mgm. per cent. In experiment 3 the glucose caused a 15-minute depression, during which the blood sugar was lower than in experiment 2, were no inhibition occurred. Whatever the cause of the occasional depression may be, surely no correlation exists between the blood sugar value and the gastric hunger activity.

In the remaining 90 per cent of the experiments, the intravenously injected glucose solution had no demonstrable effect on the gastric hunger contractions. This was true, regardless of the type of activity or the degree of tonus. In every case where the blood sugar determination was made within 20 minutes of the injection of the glucose, the blood sugar value was above 0.200 per cent. The conclusion seems inevitable that the activity of the empty stomach remains unaffected, when the blood sugar level is raised above normal by artificial means. We believe we are justified in concluding that *in normal animals the blood sugar level is not one of the controlling factors of the gastric hunger mechanism.*

Mulinos (1927) fed meat to dogs whose gastric hunger contractions were being recorded, and found that there developed an increase in gastric motility which resembled that obtained under insulin hypoglucemia. When 50 per cent glucose solution was injected intravenously into these dogs, no depression of gastric activity was ever recorded. The results may be summarized as follows: When the glucose was injected just before or immediately after the meat feeding, the high blood sugar concentration which followed had no demonstrable effect upon the promptness of initiation of the pre-digestion activity, or upon its intensity. Given at the height of the activity, which was indistinguishable from the type III hunger contractions of Carlson, the glucose proved as ineffective as when the stomach was empty.

Glucose solution injected during the quiescent period which follows the activity reported above also proved ineffective. A number of such injections were made, in order to put to test the observation of Hughson and Scarff (1924), that gastro-intestinal stimulation in a cat followed the intravenous injection of hyper-tonic glucose solution. Our failure to obtain any such stimulation in unanesthetized dogs, and the fact that Hughson and Scarff observed it but once in many experiments, suggest that hypertonic glucose solutions (50 per cent), given intravenously, usually have no stimulating effect on the quiescent stomach.

c. Insulin injected subcutaneously into normal fasting dogs, under the conditions of our experiments, elicits, in from one-half to two hours, strong gastric contractions of type III. Experiments were performed on 11 dogs, and included over 100 observations.

Under conditions of adequate diet (Mulinos, 1931) the "insulin" type of gastric tetanus appears with invariable regularity. The following protocol shows well that the degree of hypoglucemia is in some way connected with the gastric activity.

Dog 8. Weight 10.4 kilos

DATE	BLOOD SUGAR	HEMOGLOBIN	GASTRIC ACTIVITY	COMMENT
	per cent	*per cent*		
4-3-26	0.092	100	Type I	Before insulin
	0.034	108	Tetanus	30 minutes after 20 units insulin s.c.
4-8-26	0.085	100	Quiet	Before insulin
	0.053	106	Tetanus	55 minutes after 10 units insulin s.c.

These hypoglucemic reactions are extinguished by glucose, but only temporarily. That the rate of fall of the blood sugar concentration may play a part in the genesis of these contractions of the stomach is clearly shown by the following selected protocol.

Dog 4. Weight 12.0 kilos

DATE	BLOOD SUGAR	HEMOGLOBIN	GASTRIC ACTIVITY	COMMENT
	per cent	*per cent*		
4-3-26	0.095	100	Type I	Before insulin
	0.043	109	Tetanus	35 minutes after 10 units insulin s.c.
			Quiet	15 cc. 50 per cent glucose I. V.
	0.130	97	Tetanus	20 minutes after the glucose injection

This dog was studied over a period of 18 months, during which gastric activity of type III was never observed except after insulin. Therefore the tetanus coexistent with a blood sugar concentration of 0.130 per cent may be considered a true "insulin" tetanus, rather than incidental type III contractions. Under insulin, the hemoglobin concentration of the blood rises, as shown in the protocol. This is in confirmation of the findings of Drabkin and Edwards (1924). The hemoglobin values are relative, the initial determination being taken as 100.

Insulin hypermotility is abolished by atropine and by intravenous injection of glucose solution. Karo syrup and glucose by mouth also depress this activity, as they do other gastric contractions. Insulin hypermotility is uninfluenced by the ingestion of small amounts of meat.

Insulin given intravenously depresses the activity of the empty stomach and the motility of a Thiery-Vella fistula preparation of the ileum. When 10 to 30 units of insulin are injected into a vein, the activity of the stomach is completely but temporarily depressed; there is an increase in the heart rate of from 12 to 35 per cent, and in 8 experiments there was a rise in the blood sugar concentration, averaging 33 mgm. per cent. This latter has been found also by Koranyi (1930). At the same time there is momentary depression of the motility of the ileum. Both Lilly and Mulford insulins were used, with identical results. The 0.1 per cent phenol preservative has no effect upon the stomach or intestine (6 experiments). Simici, Giurea and Dimitriu (1927) studied the gastric movements of man by means of a balloon swallowed by the subject, and the emptying time by means of the x-rays. Twenty normal (human) subjects were studied. They found that immediately after 15 units of insulin intravenously there was gastric depression, followed, in about 15 minutes, by hypermotility. In our 5 dogs 48 injections resulted in gastric depression which lasted from 7 to 32 minutes. Usually the motility which followed the larger doses (2 units per kilo) was of the hypoglucemic type. After the injection of smaller doses of insulin ($\frac{1}{2}$ unit per kilo) there was a return to the original type I motility in 7 to 15 minutes. The hunger activity would last 10 to 20 minutes, and was usually followed by a short, quiet period, which in turn was succeeded by insulin hypermotility.

Simici, Popesco and Dicolesco (1926) showed that insulin injected subcutaneously or intravenously increases the acidity of the stomach contents. This was confirmed by Predietschensky, Gurwitsch and Permjakow (1927), and by Heller (1932). The increased secretion is appreciable in 30 to 90 minutes. It is evident, therefore, that insulin causes an increase in both the motor and the secretory functions of the stomach. Both effects are prevented or abolished by glucose and by atropine.

After insulin, there is hypoglucemia, and a rise in the hemoglobin concentration of the blood. When the blood sugar concentration has fallen sufficiently, there are also tachycardia, salivation, and gastric hypermotility. This hypermotility of the stomach is abolished by glucose given intravenously, and also by atropine. When insulin is injected intravenously, the stomach and intestine of the unanesthetized dog are depressed temporarily, and proportionally to the dose. The intestinal depression is momentary.

d. Dogs rendered diabetic by pancreatectomy show an increase in gastric motility which resembles type III of normal dogs, and the tetanus of insulin and of feeding. It was shown, in a previous communication (1927), later confirmed by Quigley and Hallaran (1932), that normal type III activity and the digestive activity of the stomach may be distinguished from insulin hyperactivity by the fact that the former are uninfluenced

by intravenously injected glucose solution, while the insulin tetanus is depressed. It has been determined that the gastric tetanus which accompanies pancreatectomy diabetes is uninfluenced by glucose injections. This was to be expected, because of the high concentration of blood sugar already present in diabetes.

We propose to show that diabetic hypermotility of the stomach is unrelated to the disease itself, but that it is a manifestation of the coincident "starvation" which is a part of the diabetic picture. The facts considered chronologically are these. As soon after pancreatectomy as is feasible, the gastric motility is recorded. Tetanus is the rule. Loss of weight is rapid; the appetite is enormous. Insulin treatment is then instituted, and the diet so arranged that the urine is kept sugar free. Of eight such repetitions in one animal, the blood sugar was reduced to below 0.150 per cent, and the urine rendered sugar-free within 36 hours of the institution of the antidiabetic measures. Gastric tetanus persisted, however, for from 3 to 7 days, gradually to return to the mixed type of pre-pancreatectomy. Upon withdrawal of the insulin, the blood sugar mounted immediately, with much glucosuria and loss of body weight. The hyperglucemia followed immediately the omission of a single dose of insulin. Gastric hypermotility of the diabetic type did not appear, however, until after from 5 to 11 days. It is evident that the immediate changes wrought by the diabetic state are not responsible for the gastric tetanus.

Discussion. The activity of the stomach may be increased by starvation, by insulin hypoglucemia, by pancreatectomy diabetes and in natural diabetes, by small amounts of meat put into the stomach, and by the intravenous injection of hypertonic sodium chloride or sodium sulphate solutions (denied by Quigley and Hallaran). All of this increased activity, together with the normal hunger contractions of the stomach, is inhibited by atropine or epinephrine. The food hyperactivity is temporarily inhibited by choline injected intravenously (1926). Only the insulin hypermotility is inhibited by intravenously injected glucose solution. Insulin itself injected intravenously temporarily inhibits the activity of the stomach and ileum.

The intravenous injections of hypertonic solutions of glucose or of salt result in a dilution of the blood by 3 to 12 per cent. On the contrary, insulin hypoglucemia renders the blood more concentrated, the hemoglobin per cent increase being often as much as 10. Hypertonic glucose solution had no effect on the gastric hunger activity, while hypertonic salt solution of about 16 to 20 per cent stimulated the intestines and stomach, yet both of these hypertonic solutions decrease the hemoglobin concentration of the blood. In the opposite direction, insulin hypoglucemia stimulates the stomach, and also the intestine, but is coincident with a concentration of the blood. We are forced to the conclusion that the changes in hydration

indicated by variations in the blood hemoglobin concentration have no etiologic connection with the activity of the gastro-intestinal tract. Furthermore, during severe hemorrhage in cats under amytal anesthesia, there is dilution of the blood, hyperglucemia, and increased gastric motility (Mulinos, 1928).

Starvation and diabetes have much in common, in that they are both associated with changes in metabolism and loss of weight. Our experiments point definitely to a correlation of these two conditions as regards the increased motility of the stomach. At least the changes of glucosuria, hyperglucemia and loss of weight which follow the withdrawal of the insulin have no immediate effect upon the gastric motility. It is days later that the so-called "diabetic" hypermotility appears.

We believe that insulin hypermotility of the stomach runs parallel to the effects of that substance on the central nervous system. Besides the evidence adduced in this communication is that of a previous report in which it was found difficult or impossible to produce gastric tetanus with insulin (Mulinos, 1931). In this case the dogs were resistant to insulin, several times the usual convulsant dose being necessary to induce ataxia. Furthermore, pithed cats maintained by artificial respiration show no increase in the motility of the stomach after insulin (32 experiments).

SUMMARY 1. The motility of the empty dog stomach is uninfluenced by physiological (spontaneous) variations in the blood sugar, or by raising the blood sugar concentration by intravenous injection of glucose solutions.

2. Insulin hypoglucemia is accompanied by gastric hypermotility. This motility is depressed by glucose administered by mouth or by vein.

3. Insulin injected intravenously depresses the normal activity of the stomach and ileum. There are also tachycardia and hyperglucemia. All the effects are temporary.

4. For reasons discussed in the text, the gastric hypermotility of starvation and of diabetes are considered to be analogous.

5. Epinephrine temporarily and atropine lastingly depress the normal gastric activity, and the hyperactivity which follows food, salt solution injections, diabetes, starvation, and insulin hypoglucemia.

CONCLUSION

The metabolism of carbohydrates and the glucose level of the blood appear to have no direct connection with the motor activity of the stomach.

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CHANGES IN THE SKELETAL MUSCLE OF THE RAT FOLLOWING DENERVATION

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It is well known that denervation of skeletal muscle is followed by marked changes in the weight, chemical composition, and physiological properties of the affected muscles. Many inconsistencies are to be found occurring in the various reports on the chemical changes found in such muscles. We believe that these inconsistencies are, in a large part, due to the fact that proper attention has not been given to the source of material, especially as regards the stage of the atrophy process at the time of analysis. The latter is significant because it must be remembered that any analysis made upon skeletal muscle represents the analysis of an organ and not of a single tissue. It is an analysis of skeletal muscle cells plus a variable and unknown amount of other tissue such as connective, nerve, smooth muscle, and endothelial. Further, it is well known that the ratio of muscle tissue to other tissue is greatly altered during the course of atrophy, the amount of the alteration depending upon the length of time of the atrophy.

The work herein reported represents a study of the changes which occur in muscle subsequent to denervation. In this study the rat was used as the experimental animal, thereby making available a large number of animals in which the genetic and nutritional factors were more easily controlled. One limb was denervated, the opposite limb serving as the control. The whole muscle (gastrocnemius) served as the sample for analysis. This procedure made it possible to determine not only changes in the per cent composition of certain substances but also changes in the total muscle content of those substances.

The amount and rate of change, following denervation, has been determined for muscle weight, water, total nitrogen, extractable protein fractions, acid-soluble phosphorus compounds, creatine, glycogen, potassium and calcium. The extent of change was determined by comparison with the control muscle from the same animal. Observations were also made upon the histological changes and upon the reaction of denervated muscle to electrical stimulation.

METHODS. One gastrocnemius muscle of each rat was denervated by

removing a section of the sciatic nerve about 1 cm. in length. The incision was made at the level of the trochanter. This operation was performed under light ether anesthesia. In order to avoid possible errors due to differences in the weight of opposite muscles an equal number of operations were made upon right and left legs. Changes in muscle weight due to growth were avoided by using adult animals. At designated times after operation some of the animals were killed, by a sharp blow upon the head, and bled from the carotids. The gastrocnemius muscles were carefully dissected out, freed from adherent fat, nerve, and superficial connective tissue and weighed. In preparing material for such determinations as glycogen and acid-soluble phosphorus the animals were placed under light amytal anesthesia and the gastrocnemius muscle quickly excised. This procedure avoided the changes in these tissue constituents which might occur during the time required for a careful dissection. The total amounts of glycogen and phosphorus compounds calculated to have been lost from such muscles were based upon the average weight loss values obtained from the entire group of animals.

The water content was taken to be equal to the loss in weight due to drying to a constant weight in an electric oven at 103°C.

Creatine was determined according to the method of Rose, Helmer and Chanutin (1927).

In the potassium determinations, the muscles from ten animals were pooled, dried, and a Neumann wet ash made. Aliquots of the ash were transferred to platinum crucibles, dried and analyzed according to Shohl and Bennet (1928). The titration method was employed.

Calcium was determined by the method described by Dixon, Davenport and Ranson (1929). The gastrocnemius muscles from several rats were pooled for each determination.

Glycogen was determined according to the technic of Cori (1932). In order to avoid possible errors due to incomplete precipitation of small amounts of glycogen, approximately equal weights of normal and denervated tissue were taken for analysis.

The proteins were fractionated according to a procedure described by Howe (1924). The pooled muscles of from three to five rats were used in the determination of each fraction. In some instances micro extractions were made. In such instances the pooled muscles of from two to three rats sufficed for the series of fractions made, aliquots of the mixed muscle hash being taken for the determination of each fraction. In the latter instances the nitrogen was necessarily determined colorimetrically. The nitrogen extracted from the muscle by five per cent trichloracetic acid was taken to be the non-protein nitrogen.

The trichloracetic acid filtrates of muscle for phosphorus determinations were prepared according to the procedure described by Cori and Cori

(1931), except that our extractions and filtrations were made in a cold room at 3°C. All phosphorus determinations were carried out by the colorimetric method of Fisk and Subbarow (1925). The calculations for the various phosphate fractions were based on the assumption that the total volume of the solution was equal to the volume of trichloracetic acid added plus a volume of water equal to three-fourths of the weight of the muscle. In experiments on muscles from animals subjected to the longer periods of atrophy it was occasionally necessary to add inorganic phosphate to the fraction in question in order to make the color of the unknown approximate that of the standard. The total acid-soluble phosphorus was prepared for analysis by wet ashing an aliquot of the trichloracetic acid extract with sulfuric and nitric acids. The inorganic phosphate and phosphocreatine were prepared and analyzed according to Fisk and Subbarow (1929). The additional phosphate liberated by heating in a boiling water bath for seven minutes with normal sulfuric acid, over that liberated in one-half hour with normal sulfuric acid at room temperature is taken to represent two-thirds of the phosphorus of adenosinetriphosphate which is the so-called pyrophosphate fraction. The remaining one-third is more resistant to hydrolysis and is liberated in the digest for total phosphorus. The difference between the total acid-soluble phosphorus and the total amount liberated by heating in a boiling water bath for seven minutes with normal sulfuric acid includes, therefore, one-third of the phosphate of adenosinetriphosphate and the "undetermined fraction." The latter includes hexosemonophosphate and other unknown compounds in small amounts. In tabulating the results an amount of phosphorus equal to one-half of the pyrophosphate was subtracted from the "undetermined fraction" and added to the pyrophosphate fraction to give what is called the adenosinetriphosphate fraction.

Results. *Rate of atrophy.* The rate of atrophy of the denervated muscle was a much more rapid and uniform process than has been observed for any other species. The data (table 1) concerning the weight loss are the result of experiments on 120 animals, made at periods of three, seven, fourteen, twenty-one, twenty-eight, and forty-two days after denervation. It is to be remembered that in every muscle there is a certain quantity of material, chiefly connective tissue, which is lost at a very slow rate, if at all, during a length of time comparable to that of our studies. There seems to be no method for determining the absolute amount of such material which is present in any given muscle. From our own observations, and those of others, it would appear that from ten to twenty per cent of the wet weight of normal muscle is composed of such material. We have assumed, for the purpose of calculation, that the amount of muscle substance originally available for loss amounted to 85 per cent of the weight of the control muscle. With this assumption, it can be calculated from

these data that the loss of muscle weight in any given period of time was a constant fraction of the amount available for loss. That is, the weight loss followed rather closely the curve of the equation for a reaction of the first order.

Physiological behavior. Some animals developed trophic ulcers on the denervated foot. It was found that the occurrence of these lesions was less frequent when the animals were kept on a smooth, saw-dust covered floor in a cage whose walls did not invite climbing. The number of animals which had to be discarded because of this condition was very small.

The peripheral end of the nerve failed to respond to faradic stimulation after a period of from forty-eight to seventy-two hours after denervation. This period of viability for the sectioned nerve agrees with that found by Cook and Gerard (1931) for the sciatic nerve of the dog.

Typical fibrillary contractions of the denervated muscle were present as early as the third day and persisted throughout the periods of observation.

TABLE 1

The effect of denervation upon the water content and weight of muscle

	DAYS AFTER DENERVATION					
	3	7	14	21	28	42
Per cent of weight lost	8.4	22.3	48.3	61.9	72.3	79.0
P.E.M.	±0.64	±0.84	±0.58	±1.05	±0.92	
Water content—control	75.42	75.66	76.43	75.67	75.56	75.57
P.E.M.	±0.08	±0.08	±0.19	±0.07	±0.08	
Water content—atrophy	75.42	76.34	77.31	75.72	77.37	76.33
P.E.M.	±0.06	±0.05	±0.25	±0.16	±0.33	

The denervated muscle exhibited the typical reaction of degeneration as early as the third day and continued to do so throughout the entire period of observation. The increased irritability of the atrophic muscle to the galvanic current was more apparent when the tests were made through the skin than when the stimulus was applied directly to the exposed muscle.

Histological changes. Histological studies of muscle were made at various times after denervation. These studies indicate that the microscopic changes in these denervated muscles were typical of those of denervation atrophy. The nuclei and cytoplasm appeared to stain normally with hemotoxylin-eosin. Slight inflammatory reactions were noted, especially in the earlier periods, but they appeared to be most marked about the degenerating nerve fiber. An occasional muscle fiber was undergoing phagocytosis by polymorphonuclear leucocytes. The muscle fibers became very much smaller as the time following denervation progressed.

This change resulted in a marked condensation of nuclei and connective tissue. There was no evidence for the formation of new connective tissue or the regeneration of muscle fibers. The striations of the muscle fibers were found to persist for four weeks, which is the longest period for which histological studies were made. It appears that the major change in these muscles was one involving a loss of cytoplasm from the muscle fibers and therefore a relative increase in the mass of nuclei and connective tissue.

Water content. The water content of the denervated muscles was slightly greater than that of the controls in forty-six out of sixty experiments (table 1). This finding agrees with those of Audova (1921) and of Chen, Meek and Bradley (1924) for denervated rabbits' muscle. This result is significant in connection with our studies only because it indicates that the changes in the concentration of the various substances must be regarded as real and not apparent changes due to an increased water content.

TABLE 2

The effect of denervation upon the calcium and potassium content of muscle

(Milligrams per 100 grams of wet tissue)

TIME AFTER DENERVATION	CALCIUM		POTASSIUM	
	Control	Atrophy	Control	Atrophy
days				
3			381	359
7	18.2	24.6	350	313
14	18.5	27.6	358	316
21	14.6	29.1	330	288
28	15.3	35.2	387	242

Calcium. The values for the calcium content of normal muscle (table 2) were obtained by an acid extraction method but agree well with those of Haury (1930) which represent an analysis of an ash of the whole muscle. The calcium content of atrophic muscle was found to be consistently higher than that of its control. These differences were noted to be greater in the longer periods of atrophy. No information is available as to which of the tissues contain this extra calcium, nor as to its chemical nature. It is possible that these differences may be in part accounted for by changes in the relative amount of muscle cytoplasm and nuclear material. It may be that the calcium exists in muscle in such a form that it can not be removed at the same rate as such substances as nitrogen, potassium and phosphate. It is possible that the increased concentration of inorganic phosphate in the atrophic muscle (table 3) may have contributed to a calcium deposition. Increased concentrations of calcium are often found in tissues undergoing retrogressive changes but the factors having to do

with its accumulation in such tissues are not well understood. In view of our lack of information as to the nature of the tissue calcium it would be futile to speculate as to whether our findings should be interpreted as indicating a disturbance in the antagonistic ion balance existing within the muscle.

Potassium. The concentration of potassium in the denervated muscle was found to be lower than that of its control (table 2). The differences were found to be greater in the longer periods of atrophy. The rate of potassium loss from the muscles undergoing atrophy paralleled very closely

TABLE 3

The effect of denervation upon the average content of creatine and the various acid-soluble phosphorus compounds of muscle

(Milligrams per 100 grams of wet tissue)

TIME AFTER DENERVATION	CREATINE		PHOSPHATE EXPRESSED AS MILLIGRAMS OF PHOSPHORUS									
			Total acid-soluble		Inorganic		Phospho-creatine		Adenosine triphosphate		Undetermined fraction	
	Control	Atrophy	Control	Atrophy	Control	Atrophy	Control	Atrophy	Control	Atrophy	Control	Atrophy
3 days	496	484										
P.E.M.	±8.0	±7.1										
7 days	460	423	163	154	34	36	50	39	60	58	20	22
P.E.M.	±5.1	±7.0	±7.8	±8.6	±1.7	±1.1	±4.2	±3.6	±1.7	±2.9	±1.7	±1.9
14 days	469	349	157	129	33	36	44	25	59	44	24	24
P.E.M.	±6.9	±10.9	±1.8	±3.8	±1.7	±1.3	±2.2	±1.1	±2.6	±2.6	±1.9	±2.4
21 days	472	300	152	110	33	42	41	17	56	38	22	14
P.E.M.	±5.3	±6.6	±4.6	±4.8	±3.4	±0.8	±2.0	±2.3	±3.9	±4.3	±1.5	±1.9
28 days	440	275	160	96	35	39	42	13	61	32	23	12
P.E.M.	±4.7	±2.8	±1.7	±2.8	±1.5	±1.8	±1.7	±0.7	±1.4	±2.6	±0.9	±1.5

the rate of loss of muscle cytoplasm. It appeared as if the denervated muscle had lost a certain amount of cytoplasm and the amount of potassium lost was the amount normally contained in the amount of cytoplasm lost. The lower concentration of potassium in the atrophic muscle can be attributed to a relative increase of connective tissue, which contains less potassium than does muscle tissue.

Acid-soluble phosphorus compounds. The inorganic phosphate disappeared from the denervated muscle at a rate slower than that of the weight loss. Its concentration was slightly higher in the atrophic muscles than in their normal controls (table 3). This finding is open to several inter-

pretations. If one assumes that the extra calcium found in denervated muscle exists as a form of tricalcium phosphate and corrects the observed inorganic phosphate values for the amounts thus required, then one finds that the remainder of the inorganic phosphate has disappeared from the muscle at a rate comparable to that of the weight loss. Another possibility exists, namely, that a large part of the determined inorganic phos-

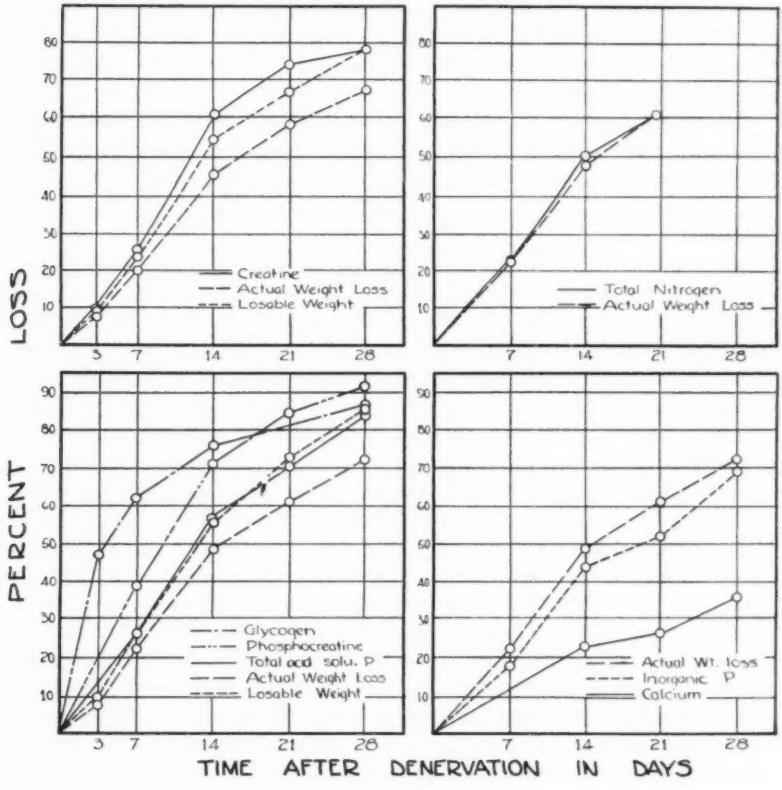

Fig. 1

phate had its origin from the hydrolysis of phosphocreatine. It is generally held that the inorganic phosphate values for muscle as determined are higher than those actually existing in the living muscle because of the ease with which phosphocreatine undergoes hydrolysis. The utmost precautions were taken to maintain identical conditions in the analysis of the atrophic muscle and its control. However, the presence of fibrillary con-

tractions in the denervated muscle probably led to a greater degree of phosphocreatine hydrolysis.

The phosphorus determined as belonging to phosphocreatine was found to have disappeared at a much faster rate than that of the weight loss (fig. 1). The fall in the phosphocreatine concentration in denervated muscle (table 3) is too great to be accounted for by the changes in the relative amounts of muscle and connective tissue. These changes were noted as early as one week after denervation and are similar to those observed by other investigators for other species. We believe that the fibrillary contractions are largely responsible for the low concentration of this substance in denervated muscle.

The adenosinetriphosphate fraction disappeared from the denervated muscle at a rate which corresponds closely to that of the weight loss. The fall in the tissue concentration of this substance can be ascribed to changes in the relative amounts of muscle and other tissues in the denervated muscle.

The "undetermined" phosphate fraction, which includes hexosemonophosphate, was found to be present in lower concentration in the longer periods of atrophy (table 3). Since this fraction was determined by the method of difference, it is probable that the errors in its determination are greater than for the other fractions and the differences between the denervated and control muscle concentration of this fraction are less significant. However, it is apparent that this fraction behaves similarly to the adenosinetriphosphate fraction.

The total acid-soluble phosphorus, the sum of the above four fractions, was found to have been lost at a rate quite comparable to that of the weight loss, the greatly decreased concentration of phosphocreatine being balanced by the increased concentration of inorganic phosphate.

Creatine. The creatine content of muscle was studied in experiments upon forty animals at periods of from one to twenty-eight days after denervation. As early as the third day, and thereafter, the denervated muscle was found to have lost creatine (fig. 1). The concentration of creatine in the denervated muscle was lower than that in its control within one week after denervation (table 3). The rate of creatine loss followed that of the weight loss and the lowered concentration is to be accounted for by the alteration in muscle tissue and connective tissue ratio in the atrophying muscle.

Nitrogen. The values for total nitrogen show no significant variations from the control values (table 4). A change in the relative amounts of muscle and connective tissue would not be expected to alter this value. The total extractable nitrogen and the various nitrogen fractions which were extracted with different strengths of phosphate solution maintained practically a constant per cent composition, as compared to their controls,

during the atrophy of denervation. This indicates that these various fractions, whether or not they represent true individual proteins, are lost at approximately equal rates during atrophy. These findings differ from those of Streyrer (1904), who, using the methods outlined by von Furth (1895), found evidence for an altered myosin-myogen ratio in denervated muscle. Since the von Furth fractionation depends on a graded heat coagulation and since the pH of the extract has a marked influence on the amount of protein which will coagulate at a given temperature, the results of Streyrer might well be due to a difference in the rate of acid production in the extracts, especially since the von Furth extraction fluid is unbuffered.

The non-protein nitrogen, which was determined upon a five per cent trichloracetic acid filtrate, had essentially the same concentration in normal and atrophic muscles. However, since two substances, creatine and

TABLE 4

The effect of denervation upon the nitrogen content of muscle

(Grams of nitrogen per 100 grams of wet muscle)

	7 DAY ATROPHY*		14 DAY ATROPHY*		20 DAY ATROPHY†	
	Control	Atrophy	Control	Atrophy	Control	Atrophy
Total nitrogen	3.66	3.62	3.67	3.50	3.56	3.55
Total extractable N_2	1.33	1.34	1.31	1.19	1.37	1.25
1.125 M phosphate extractable N_2	0.89	0.96	0.77	0.77		
1.725 M phosphate extractable N_2			0.51	0.54		
2.025 M phosphate extractable N_2	0.53	0.51	0.45	0.44	0.62	0.56
5 per cent trichloracetic acid extractable nitrogen	0.36	0.32	0.32	0.35	0.33	0.34

* Data were obtained by the macro method.

† Data were obtained by the micro method.

adenosinetriphosphate, which would contribute to this nitrogen fraction, were found to be present in lower concentrations in the atrophic muscle, it would seem that some of the other nitrogenous substances which would be present in this fraction must have been present in increased amounts. In this connection it is interesting to note that Chen, Meek and Bradley (1924) found no significant differences in the amino acid content of normal and atrophic muscles of the rabbit, but did find a greater amount of tyrosine reacting fragments in the latter.

Glycogen. Glycogen studies were made at intervals of three, seven, fourteen and twenty-eight days after denervation (table 5). In all cases the glycogen content of the denervated muscle was lower than that of its control. Three days after denervation the glycogen content of the atrophic muscles averaged forty-three per cent less than their controls. The differ-

ences were even greater in the longer periods. The loss of glycogen occurred earlier and to a greater extent than that of any of the other substances studied. Other investigators have found an increased glycogen content in denervated muscles from other species. It is not known whether these findings represent a species difference. Diet, previous activity, and the mode of killing might affect differently the glycogen content of normal and denervated muscle. We have endeavored to remove the tissues under conditions which permitted a minimal disturbance of its glycogen stores.

Fat. No studies were made upon the fat content of the muscles. The finding of approximately normal values for the water content and the nitrogen content of the atrophic muscles excluded the possibility of any appreciable changes in the fat content of such tissues.

TABLE 5

The effect of denervation upon the glycogen content of skeletal muscle

(Milligrams glycogen per 100 grams of muscle)

3 days*		7 days*		14 days*		28 days*	
Control	Atrophy	Control	Atrophy	Control	Atrophy	Control	Atrophy
587	324	533	313	476	205	615	215
433	306	520	274	445	178	554	266
549	261	510	214	629	278	499	286
		419	214	565	280	444	291
				528	206		
				614	319		
Average 523	297	496	254	543	244	528	265

* After denervation.

Discussion. The rat was found to be a very satisfactory animal upon which to study the problems concerned with the atrophy of denervation. Our findings are, in many respects, similar to those made on other species. The microscopic studies, the occurrence of fibrillary contractions and the presence of the reaction of degeneration all indicate that we are dealing with a process that is similar to the atrophy of denervation in other species.

The rate of weight loss is more rapid than has been observed for any other species. It seems that the rate of weight loss in the denervated muscle may bear some relationship to the growth rate of the species. The studies that have been made upon the rate of atrophy of corresponding muscles of the rat, rabbit, dog and human would indicate that for these species, at least, there appears to be a direct relationship between per cent loss of muscle weight in a given time and the growth rate.

The chemical studies were planned to determine not only the per cent

change in composition but also to permit the calculation of the total amount of change in the various muscle constituents for any designated period.

The substances studied fell into four general groups on the basis of their rate of loss as compared to weight loss. One group was lost at a rate very nearly equal to the weight loss. That is, the per cent composition remained the same during the course of atrophy. In the second group the per cent composition was slightly lower than the control, in the third group the per cent composition was markedly lower than the control, and in the fourth there was an increase in concentration of the substances in the denervated muscle.

In the first group belongs the total nitrogen, extractable nitrogen fractions, and non-protein nitrogen. Since the water content changes only a very slight amount, the total nitrogen would not be expected to change appreciably since the per cent nitrogen in connective and muscle tissue must be of the same order when they have equal water contents. It might be expected that as the organ gradually comes to contain relatively more connective tissue, the phosphate extractable nitrogen would decrease. It is not known what portion of these fractions come from muscle tissue and what from connective tissue. The possibility exists that the muscle tissue protein actually becomes more soluble as the atrophy progresses, the relative increase in the less soluble connective tissue proteins masking the change. The constancy of the non-protein nitrogen is apparently happenstance for it is known that two substances contributing to this fraction are lowered in concentration. This means that certain other substances, the nature of which is unknown, have increased to the same extent.

The second group of substances includes creatine, adenosinetriphosphate, potassium, and total acid-soluble phosphorus. It will be noted that the decrease of concentration in these substances has occurred at almost exactly the same rate and to approximately the same extent. This leads one to look for a given factor in the process of atrophy which could be expected to lead to such a finding. The explanation probably lies in the fact that only muscle tissue cytoplasm was lost from the muscle in appreciable amounts. The consequent increase in the relative amount of connective tissue must lead to a lowered concentration of any substances which are present in appreciably greater amounts in the muscle cytoplasm. This was undoubtedly true for all the substances classed in this group. In fact, if we assume that 85 per cent of the weight of the control muscle represents the total original weight of substances which might be lost from the atrophied muscle, and then calculate the weight loss in the percentage of this calculated "losable weight" it is found that the loss of weight of this fraction coincides very closely to the loss of weight of any of the substances

in this group. This means that the explanation for the rate of loss of these substances is to be found in the explanation of the rate of loss of the cytoplasm as a whole.

Only two substances were found to decrease in concentration at a rate such that their total loss, in a given time, would be markedly greater than the weight loss for that time. These were phosphocreatine and glycogen. It would be expected that their rate of loss would be at least as great as the rate of weight loss. It was, in fact, much greater and cannot be accounted for by the changes in the relative amounts of muscle and connective tissue. Both of these substances are very closely associated with the contraction process of muscle. It is an established fact that their concentrations are reduced in an active muscle as compared to a resting control muscle. We believe that this marked loss of both of these substances, from denervated muscle, is due to the fact that the so-called resting muscle is in reality an active muscle at the time of its removal for analysis. The constant fibrillary contractions of the denervated muscle precludes the possibility of obtaining the atrophic muscle for analysis in a condition even approximating a resting state. The explanation for this finding seems then to rest upon the explanation for the fibrillary contractions. In the frog, in which fibrillary contractions following denervation were not observed (Langley, 1915), no decrease in phosphocreatine concentration was observed until several weeks after denervation (Moschini, 1932). The work of others has pointed out that the values for the phosphocreatine content of muscle are necessarily minimal because of its ease of hydrolysis. It is clear that the potential phosphocreatine of these denervated muscles, as indicated by the creatine and inorganic phosphate values, was much higher than the observed phosphocreatine and the loss of the potential phosphocreatine follows very closely the "losable weight" loss.

Inorganic phosphate, calcium, and the "rest non-protein nitrogen" were found to increase in concentration in the denervated muscle. If it is assumed that the excess calcium is present in the form of tricalcium phosphate or some of its compounds and the amount of inorganic phosphate so required is subtracted from the total phosphate, then the remainder of the inorganic phosphate is found to have been lost at the same rate as the "losable" weight loss. Not more than a small fraction of this extra calcium can be accounted for by the change in muscle connective tissue ratio. No satisfactory explanation can be given, at the present time, for this increase in the muscle calcium.

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SUMMARY

A study has been made of the atrophy of denervation in the rat's gastrocnemius muscle. Chemical, microscopic and physiological studies have

been made upon the denervated muscle at various periods from 3 to 42 days after nerve section. The rat was found to be a very satisfactory animal upon which to study this problem. The rate of atrophy was found to be exceedingly rapid and uniform. The loss of muscle weight in any given period of time was a constant fraction of the amount available for loss.

The microscopic changes were similar to those described for other species. The presence of fibrillary contractions and a typical reaction of degeneration was noted as early as three days after denervation. By employing the entire muscle as a sample for analysis it was possible to determine per cent changes in composition and to calculate the amounts of the various substances that had been lost from the denervated muscle during any designated period.

Little change was noted in the water content of the denervated muscle. It was found that creatine, total acid-soluble phosphorus, adenosinetriphosphate, total nitrogen, extractable nitrogen and potassium were lost from the denervated muscle at a rate which closely paralleled that of the weight loss. Extractable calcium and inorganic phosphate were found to have disappeared at a much slower rate than that for the weight loss. An early and marked lowering of glycogen and phosphocreatine content was observed in the atrophic muscle. It is believed that the fall in concentration of the above substances in the denervated muscle can be attributed to the effects of the activity of fibrillary contractions and a relative increase in the amounts of non-muscular tissue in which these substances are absent or present in lower concentrations.

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THE RELATION BETWEEN BLOOD OSMOTIC PRESSURE AND INSENSIBLE WEIGHT LOSS

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The relation of the insensible weight loss of the body to its total metabolism has in recent years become a subject of primary interest and importance. Rubner (1902), Benedict and Carpenter (1910), Gephart and DuBois (1916), Soderstrom and DuBois (1917) all for normal men, showed that a definite proportion (around 24 per cent) of the total heat produced by the body is lost by vaporization of water from the skin and lungs. Benedict and Root (1926) used this method of measuring the insensible weight loss as a means of determining total metabolism. In the study of human metabolism the validity of this method for the estimation of total calories produced has been confirmed by the observations of Levine, Kelly, and Wilson (1930) and Johnston and Newburgh (1930). The measurement of insensible weight loss has also been suggested as a method for determining the metabolism of smaller animals (Greene and Luce, 1931).

The extreme simplicity of this procedure for the determination of total metabolism favors its wide adoption. Therefore any conditions in which this relationship between the total calories produced by the body and the proportion lost through vaporization of water may be disturbed deserve recognition and careful definition.

One of us (H. G. B.) has recorded many observations that at the onset of fever produced by the administration of cocaine there is a concentration of the blood, as indicated by an increase in specific gravity both of the whole blood and of the serum. Recent data (Barbour and Gilman, 1932) have demonstrated a concomitant increase in osmotic pressure of the serum. Coincident with this blood concentration is the well-known increase in the total metabolism. However, a simultaneous measurement of the rate of insensible weight loss at a time when the animal exhibits a definite increase in metabolic rate reveals that *the calories lost through vaporization of water from the lungs and skin remain the same or are even diminished despite the increased heat production* (Marshall, Aydelotte and Barbour, 1931). This inability to meet the demand of an augmented heat production by an

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increased vaporization of water is believed to be fundamental in the production of fever. An explanation of the failure of the organism to dissipate its excess heat in the form of increased water of vaporization would seem to lie in the increased concentration of the circulating fluid. Consequently the experiments to be reported in this paper were conducted to determine the effect of variation in the concentration of blood electrolytes upon the insensible weight loss of the body.

Search of the literature reveals an extensive controversy in regard to the effect of water administration upon the insensible weight loss. Erismann (1870) was apparently the first to notice the relationship; Schwenkenbecher (1904), on the other hand, reported that the administration of large quantities of water at 15 to 20° had no effect upon the rate of elimination of water from the skin. His work is confirmed by Jores (1930), and opposed by Moog and Nauk (1921) and others. Careful consideration of the above papers reveals omissions and discrepancies in technique which may account for the opposing results. In no case was any measure made of the actual dilution of body fluids produced by water administration. The amount of fluid administered by various investigators differed tremendously, as did the temperature of the fluids when given. The necessity of temperature control will be emphasized later in this paper.

A recent and significant contribution to the relation of body hydration to insensible weight loss is that of Manchester, Husted and McQuarrie (1931). They observed in a normal ("saturated") state of hydration "a close parallelism between the insensible loss and energy metabolism under normal conditions." However, they dehydrated some of their subjects by means of special diets and water restriction. Under such conditions the insensible weight loss was less than that predicted from the total metabolism. Conversely when large amounts of fluid were administered the opposite was true.

The above mentioned work would seem to indicate that the rate of insensible loss is a function not only of the total metabolism but of the state of body hydration. While the rate of surface water loss can be readily determined its immediately controlling factors have never been established. In view of the evidence of a relation to the state of hydration of the body as a whole the situation seems first of all to demand simultaneous observations on the rate of water loss and the osmotic pressure of the blood; for the osmotic pressure of the circulating fluid should reflect the degree of body hydration. Osmotic pressure observations would seem of vital importance to the deductions of Manchester, Husted and McQuarrie who made no such accurate measure of the degree of hydration or dehydration of their subjects.

EXPERIMENTAL METHODS AND PROCEDURE. Rabbits weighing approximately 2.5 to 3.0 kilos were used as the experimental subjects in this study.

In order to determine their insensible weight loss the animals were placed in cylindrical, wire-mesh, false-bottom rat cages. Thus they received ventilation from all sides, vaporization from the skin being unimpeded. The size of the cage was such as to allow comfort to the animals and yet restrict their movements so as to assure basal conditions. The rabbit, in the cage, was weighed upon a Becker chainomatic balance capable of weighing 5 kgm. to within less than 1 mgm. When weighing the animals, however, their rate of weight loss was too rapid to permit such accuracy. The procedure adopted was to select a weight less than, but within 50 mgm. of the animal's actual weight and record within 30 seconds the time at which the subject reached this selected weight. Determinations were made over intervals of 20 minutes or more, the rabbits remaining in their cages between weighings.

After three periods were obtained during which the rate of insensible weight loss remained constant, the figures so obtained were accepted as the basal level, and the animals were then subjected to the experimental procedure. A normal sample of blood was drawn from the left ventricle, after which either 35 cc. per kilo of 5.0 per cent NaCl or 75 cc. per kilo of water were administered by stomach tube. Control animals received equal volumes of isotonic NaCl. After the administration of these fluids no measurements were made for at least 30 minutes in order to allow the subjects to regain their basal state after the manipulative procedure. Determinations of the rate of insensible weight loss were then continued over periods of at least 20 minutes for several hours. At various intervals osmotic pressure determinations were made upon the blood. The vapor pressure method of Hill (1930) as described in detail by Margaria (1930) was used to measure the blood osmotic pressure. We have found the error of this method to be no greater than ± 0.5 per cent.

One precaution essential in metabolic work involving the administration of large quantities of liquid has apparently been neglected in previous studies of the insensible weight loss; this is the temperature of the fluid when given. If one administers the liquid at body temperature, the effect of this warm water plus the metabolic stimulus which must accompany the passage of a stomach tube in a rabbit increases the number of calories lost through water vaporization from the skin and lungs. We have found the optimum temperature for this fluid when administered to rabbits to be about 25°C. If one gives liquid at this temperature the excess calories produced by its administration are roughly balanced by the cooling action of the fluid several degrees below body temperature. By compensating errors in this fashion the animal soon returns to "normal." However, in all our experiments, the administration of either hypertonic saline or water was always controlled by giving isotonic saline to other animals, so that regardless of the temperature effects of the experimental solutions their

action upon the rate of insensible weight loss could be ascertained by comparison.

EXPERIMENTAL RESULTS. The results of the administration of hypertonic saline are illustrated in figure 1. The change in rate of insensible weight loss is expressed as per cent deviation from the figure obtained in the normal basal period. A glance at the chart will show that in every case the rate of loss of the experimental animals was appreciably less than that of the controls. The most extreme experiment shows a decrease of over 50 per cent. This was coincident with an approximate increase of 20 per cent in the osmotic pressure of the blood.

Figure 2 on the other hand illustrates the opposite procedure. These observations were made upon experimental animals receiving water, as

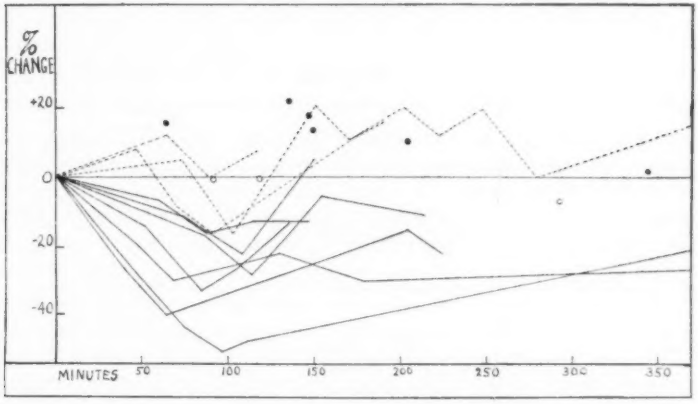

Fig. 1. Effects of hypertonic saline given per os in normal rabbits. Ordinates: per cent change; abscissae: time in minutes; solid dots: serum osmotic pressure change in experimental animals; circles: serum osmotic pressure change in control animals treated with isotonic saline; continuous lines: insensible weight loss in experimental animals; broken lines: insensible weight loss in control animals treated with isotonic saline.

contrasted to the controls which received an equal amount of isotonic saline. The serum osmotic pressure of the control animals remained the same throughout the experiment. Their rate of weight loss also remained fairly constant. The experimental subjects, run at the same time under identical environmental conditions, are in sharp contrast. Following the administration of water the osmotic pressure of their blood showed a decrease of about 10 per cent. As a result of this dilution of body fluids the skin and lungs were instrumental in ridding the body of excess fluid to the extent that in one case the rate of insensible weight loss was 60 per cent

greater than that observed during the basal period, and equally in excess of the rate of loss of the experimental controls. Such figures leave little

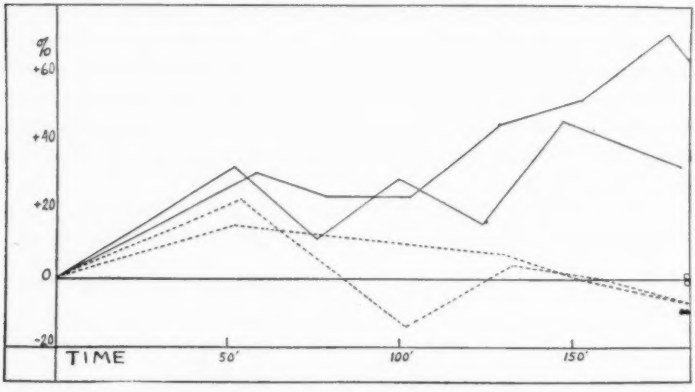

Fig. 2. Effects of water per os in rabbits. Ordinates: percentage change; abscissae: time in minutes; solid dots: serum osmotic pressure in experimental animals; circles: serum osmotic pressure in control animals treated with isotonic saline; continuous lines: insensible weight loss in experimental animals; broken lines: insensible weight loss in control animals treated with isotonic saline.

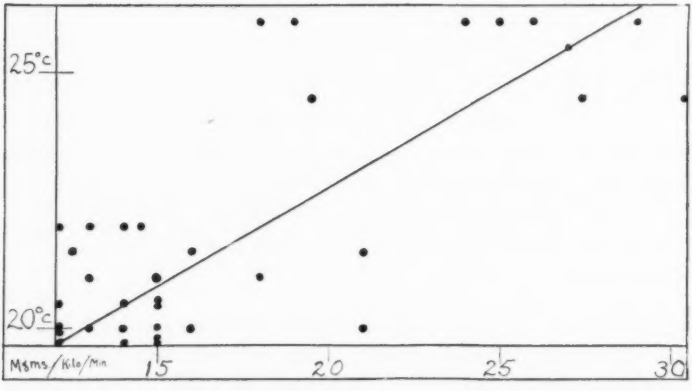

Fig. 3. Relation of insensible weight loss to room temperature during basal periods. Ordinates: degrees Centigrade; abscissae: weight loss milligrams per kilogram per minute.

doubt that the state of body hydration is one of the primary factors determining the rate of vaporization of water through the skin and lungs.

Another factor which according to our results has an extreme influence upon the rate of insensible weight loss is the temperature of the environment. Most of our observations were made at a room temperature of approximately 20°C. However, a few measurements were made when the environmental temperature was as high as 26°. The relation between room temperature and insensible weight loss in rabbits is graphically illustrated in figure 3. It will be seen that for an environmental increase from 20° to 26° the rate of insensible weight loss is almost doubled.

Discussion. It seems unnecessary to dwell upon the question as to whether or not the changes observed in the rate of insensible weight loss following the ingestion of anisotonic solutions may be merely a reflection of an altered rate of metabolism. No measurements were made of the total metabolism for it has long been known that the effects of water and sodium chlorid solutions upon the bodily oxidations are negligible. Furthermore the metabolism, if affected at all may become increased by hyper- as well as hypotonic salt solutions (Grafe, 1923). Manchester, Husted and McQuarrie (1931) report an experiment in which they measured total metabolism coincident with insensible perspiration in one of their dehydrated subjects. They found no significant influence of dehydration on energy metabolism.

With the energy metabolism remaining constant it is necessary to account otherwise for the pronounced changes in rate of insensible weight loss. The respiratory rate may be significant in this regard. We have observed (unpublished data) that in pronounced hyperpnea, produced by the administration of toxic doses of antipyretics to fevered animals, the amount of water lost through the lungs may be so great as to cause appreciable dehydration. We have also observed that following the administration of hypertonic saline the respiratory rate was lowered. The possibility exists that the osmotic pressure of the blood may influence the respiratory center, allowing the animal either to dissipate or conserve water.

The purely physical aspects of the regulatory mechanism for surface evaporation seem to afford the most satisfactory explanation of our results. The administration of salt and water will so alter the vapor pressure of the blood as to either inhibit or facilitate vaporization. Regardless of the fundamental mechanism involved, however, it is obvious that in the determination of total metabolism, as measured by the insensible perspiration of an organism, special attention must be paid to the state of hydration and the environmental temperature.

SUMMARY AND CONCLUSIONS

Measurements were made of the rate of insensible weight loss in rabbits before and after the administration of hypertonic saline and water as contrasted with control subjects receiving isotonic saline.

The extent of hydration or dehydration was determined by measurements of the osmotic pressure of the blood. An increase in blood osmotic pressure caused a marked decrease in the rate of insensible weight loss, whereas a decreased osmotic pressure of the blood resulted in an increased rate of water vaporization from the skin and lungs. Changes in the environmental temperature also greatly influenced the insensible perspiration.

While the belief that under normal conditions a definite proportion of the total heat produced by the body is lost through vaporization of water from the skin and lungs is not questioned, our results show how greatly the amount of water thus dissipated may be varied by procedures not affecting significantly the total energy metabolism.

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EFFECTS OF ADRENALECTOMY AND CORTICO-ADRENAL EXTRACT ON RENAL EXCRETION AND TISSUE FLUIDS[1]

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In our earliest experiments on cortico-adrenal extract in 1930 we observed that the injection of the material into adrenalectomized cats was invariably and quickly followed by urination. At that time we were primarily interested in the effects of the extract on carbohydrate metabolism, and the phenomenon was regarded as possibly a water diuresis. Later it became apparent that while the extract brought about urinary excretion in adrenalectomized animals, large amounts of other fluids (glucose, saline or adrenalin solutions) did not have the same effect. This brought to mind a former idea that the cortex of the adrenal glands played some important rôle in the maintenance of normal renal function.

In previous papers (Britton and Silvette, 1931) it was pointed out that the profound anhydremia in adrenalectomized animals, and the subsequent extensive changes in the circulation after the administration of extract, should be carefully investigated. It was further suggested that there were "possibly also indirect actions of the hormone, brought about by enhanced renal secretion following circulatory improvement" (1931b).

Marshall and Davis suggested in 1916 the possible interrelationship between the adrenals and the kidneys. For a time it seemed likely, from certain other recent reports of a preliminary nature from the Princeton and Johns Hopkins laboratories, that this question was about to be investigated anew (Harrop *et al.*, 1931). The desire not to trespass in the field kept us for some time from carrying on the experiments herein reported, for which we had earlier obtained such promising leads. Since no extensive or critical work on the renal-adrenal interrelationship has, however, been reported up to the present time, we have recently investigated the problem further.[2]

[1] Grateful acknowledgment is made of aid received from the Committee for Research in Problems of Sex and the Grants-in-Aid Committee of the National Research Council.

[2] Since this article was prepared for press a paper by Swingle, Pfiffner and their associates appeared in Science (lxxvii: 58, January 13, 1933). There are several features brought forward in this report which are related to our earlier investigations and those now under way, as well as to our first published theory of adrenal

METHODS. In the experiments on urinary output, male white rats weighing between 100 and 150 grams were used. We have previously demonstrated that immature or young adult rats are relatively more susceptible to adrenal removal and later extract treatment than older animals. The operation was performed in one stage, care being taken that the whole glands together with a small amount of the surrounding connective tissue were removed. A number of such bilaterally adrenalectomized animals began to lose weight immediately following the operation, in spite of a plentiful and adequate dietary; this weight loss was taken as the criterion of adrenal insufficiency. Animals which did not lose but rather gained weight after adrenal removal were not used. In all cases the rats were fasted for 12 to 18 hours before the start of an experiment; during this preliminary period water was permitted *ad lib.*

The rats were placed in small wire-mesh metabolism cages which were clamped over funnels about six inches in diameter. The funnel spouts were introduced into test tubes in which a small quantity of toluene was placed as a preservative. There was practically no evaporation of fluid from the tubes, as the close fit of the funnel and tube reduced the air circulation, and the layer of toluene over the urine also prevented volatilization. In all experiments except those dealing with the excretion of phenolsulphonphthalein (to be described later) the metabolic period was 24 hours. At the start of each experiment the animal was weighed and an initial intraperitoneal injection of 10 cc. of fluid per 100 grams of body weight was given; at the end of 6 hours a second similar injection was administered: In all, 20 cc. of material per 100 grams of rat were therefore given daily. At the end of the 24th hour the experiment was terminated; all the urine was collected, and the rat again weighed.

Before the beginning and also immediately at the end of the metabolic period, the animals were forced to empty the urinary bladder by means of gentle abdominal massage; the first small amount of urine expelled was discarded, and the end sample was combined with the total urinary output. Experience showed that massage of the abdomen was effective in removing the last traces of the urine from the bladder. After each experiment the funnels were thoroughly washed with a "rubber policeman" and a fine stream of water from a wash-bottle, and the measured washings combined with the measured 24-hour urine.

Urine chlorides were determined by a modified Volhard-Harvey titration (Peters and Van Slyke, 1932); blood and urinary urea by the manometric hypobromite method of Van Slyke (1929); and blood sugar by the

function advanced about a year ago ("The Apparent Prepotent Function of the Adrenal Glands," S. W. Britton and H. Silvette, This JOURNAL, 1932, c, 701). In view of the Princeton hypothesis we are making further inquiry into phases of cortico-adrenal activity which already appear to add support to our published conclusions.

ferricyanide method of Folin and Malmros (1929). Glycogen determinations were made according to the method described in a previous paper (Silvette and Britton, 1932).

The water content of the tissues was determined by drying the whole organ or adequate portions of it to constant weight in an electric oven at 108°C. In the experiments on cats, the liver, skeletal muscle, kidney, spleen, brain, skin (plus hair), and blood were used for water analysis. In the work on rats, the animal was killed at the end of the metabolic period, 2 cc. of blood drawn for urea and sugar determinations, and the liver removed *in toto* and placed in a covered aluminum dish, weighed and dried; the rest of the carcass with the exception of the gastro-intestinal tract was then run quickly through a meat-chopper three times, and a representative sample taken and dried in the same manner.

In the experiments on the excretion of phenolsulphonphthalein, a solution containing 0.2 mgm. per cent of the dye and 0.68 per cent of sodium chloride was used; one injection of 10 cc. per 100 grams of body weight was administered. At the end of the third and again at the end of the sixth hour the urine was collected with the precautions taken above, the volume measured and the amount of the dye excreted then determined colorimetrically against a freshly-prepared standard.

All injections were given intraperitoneally on the basis of body weight. Urea solutions in high and in low concentration, 0.85 per cent sodium chloride, glucose solutions and distilled water, either alone or in combination with cortico-adrenal extract (Britton and Silvette, 1931b), were given to normal and adrenalectomized rats. In addition, metabolic studies were performed on normal and operated rats allowed free access to water.

The cats used in our experiments were adrenalectomized in one stage by the abdominal route. When symptoms of insufficiency appeared, the animals were killed and the tissues and blood analyzed.

RESULTS. *Excretion of sodium chloride, urea and water.* Untreated adrenalectomized rats excreted more chlorides and less water than normal rats under the same experimental conditions (table 1). When large quantities of distilled water were injected intraperitoneally into adrenalectomized rats, the inability of the animals to eliminate fluid was markedly manifested, although the excretion of chlorides and urea (in mgm. per cc. of urine) under the same conditions was not significantly affected by the operation.

Injections of isotonic sodium chloride, or glucose or dilute urea solutions, elicited somewhat the same results (table 1): the adrenalectomized animal practically failed to excrete water, while the elimination of chlorides and urea, when expressed in milligrams of material per cubic centimeter of urine, was not greatly different from that observed either in normal animals, or in operated animals which received cortico-adrenal extract simultaneously with the injected material.

TABLE 1

Effects of various materials and cortico-adrenal extract on the water, chloride and urea output of normal and adrenalectomized rats

RAT NUMBER	EXPERIMENTAL CONDITIONS	EXTRACT, CC. PER 24 HOURS	ANIMAL WEIGHT		CONCENTRATION OF INJECTED MATERIAL	24-HOUR URINARY EXCRETION		
			Initial	Final		Volume	Chloride	Urea
Urea injected*								
			grams	*grams*	*per cent*	*cc.*	*mgm.*	*mgm.*
45	Normal	0	130	113	0.4	27.5	16.7	20.0
46	Normal	0	148	130	0.4	29.5	11.7	10.7
47	Normal	0	139	122	1.0	29.5	10.7	28.8
48	Normal	0	135	117	1.0	28.5	19.9	30.7
51	Normal	0	140	125	2.5	24.5	24.0	59.7
52	Normal	0	145	168	5.0	1.5	1.8	3.0
54	Normal	0	132	154	5.0	1.0	2.7	2.8
53	Normal	9.9	132	117	5.0	24.0	21.8	59.4
79	Normal	13.0	130	108	5.0	35.5	17.9	104.2
1	Adrenalectomized	0	130	151	0.5	1.0		0.1
5	Adrenalectomized	0	114	123	0.4	5.5	2.6	3.0
9	Adrenalectomized	0	153	178	0.2	1.0		0.3
10	Adrenalectomized	0	152	177	0.3	1.0		0.1
2	Adrenalectomized	12.7	127	119	0.5	21.5	14.7	18.2
6	Adrenalectomized	2.3	114	105	0.4	22.5	16.9	20.1
7	Adrenalectomized	7.1	118	106	0.4	18.5	7.8	23.0
8	Adrenalectomized	13.2	132	111	1.0	25.0	11.7	34.5
11	Adrenalectomized	12.7	127	114	2.5	28.5	20.0	64.5
NaCl injected*								
49	Normal	0	128	117	0.85	24.5	125.0	16.6
50	Normal	0	137	120	0.85	29.0	168.0	13.3
69	Normal	0	142	126	0.85	29.0	158.0	9.3
70	Normal	0	142	127	0.85	28.5	146.0	11.9
57	Adrenalectomized	0	100	107	0.85	4.7	32.1	8.2
58	Adrenalectomized	0	115	121	0.85	7.5	40.4	9.1
73	Adrenalectomized	0	95	82	0.85	10.0	46.3	9.5
74	Adrenalectomized	0	98	92	0.85	11.5	69.0	5.7
59	Adrenalectomized	10.8	108	100	0.85	24.0	128.0	21.3
60	Adrenalectomized	10.5	105	96	0.85	16.0	90.0	10.3
75	Adrenalectomized	11.2	112	104	0.85	21.0	124.0	5.8
76	Adrenalectomized	11.2	112	106	0.85	19.0	112.0	8.7

* Ten cubic centimeters fluid per 100 grams of body weight; one injection at 0 hour, second injection at end of 6th hour, experiment stopped at end of 24th hour.

TABLE 1—*Concluded*

RAT NUMBER	EXPERIMENTAL CONDITIONS	EXTRACT, CC. PER 24 HOURS	ANIMAL WEIGHT		CONCENTRATION OF INJECTED MATERIAL	24-HOUR URINARY EXCRETION		
			Initial	Final		Volume	Chloride	Urea
Water injected*								
			grams	*grams*	*per cent*	*cc.*	*mgm.*	*mgm.*
71	Normal	0	148	135		26.0	12.6	12.6
72	Normal	0	120	112		20.5	9.7	9.6
3	Adrenalectomized	0	125	135		2.0	5.0	2.1
77	Adrenalectomized	0	118	127		7.5	5.2	10.3
101	Adrenalectomized	0	109	116		4.7		
102	Adrenalectomized	0	124	133		6.2		
4	Adrenalectomized	12.0	120	109		21.5	20.9	14.9
78	Adrenalectomized	11.0	110	107		17.5	18.0	13.3
103	Adrenalectomized	13.1	131	120		19.5	19.0	10.3
104	Adrenalectomized	14.0	140	134		23.0		
No injection: Water ad lib.								
55	Normal	0	130	120		3.9	18.5	11.5
56	Normal	0	125	117		5.5	10.0	11.8
61	Normal	0	131	116		6.5	16.0	6.7
62	Normal	0	140	131		4.5	11.8	7.4
63	Normal	0	134	116		7.5	15.5	8.2
64	Normal	0	125	115		7.5	11.8	8.1
14	Adrenalectomized	0	111	102		2.5	23.4	7.7
15	Adrenalectomized	0	107	101		2.5	26.1	8.3
16	Adrenalectomized	0	115	103		6.5	15.0	10.3
17	Adrenalectomized	0	131	120		5.0	20.6	9.7
65	Adrenalectomized	0	98	85		3.6	29.7	7.6
66	Adrenalectomized	0	98	87		5.3	24.6	13.9
67	Adrenalectomized	0	112	98		4.0	31.2	4.3
68	Adrenalectomized	0	114	98		6.0	41.8	6.6

Adrenalectomized rats which were injected with urea or sodium chloride or glucose solutions or distilled water *plus* cortico-adrenal extract showed a profuse outpouring of urine, and an increased total excretion of chlorides and urea (table 1). In general, no difference in urinary excretion was observed between normal animals and operated animals receiving extract.

It was observed that normal rats easily tolerated large amounts of urea up to 0.8 gram within 24 hours. Adrenalectomized rats succumbed quickly, in contrast, after the injection of amounts as small as 0.1 gram. It took ten times as much urea—one gram or more per 100 grams of body weight—to overwhelm normal rats. When cortico-adrenal extract was

given along with urea, however, normal rats sustained apparently without ill effect as much as 1.3 gram of urea, and adrenalectomized rats similarly treated were as efficient in metabolizing urea as normal animals not treated with extract (table 1).

According to Herter and Wakeman (1899) the toxicity of urea depends on its rate of excretion by the kidney. These workers found the maximum

TABLE 2

Effect of adrenalectomy and extract treatment on the phenolsulphonphthalein excretion of rats

RAT NUMBER	WEIGHT	EXPERIMENTAL CONDITION*	URINARY EXCRETION, 6-HOUR PERIOD			
			Volume		Phenolsulphonphthalein	
			Cubic centimeter	Per cent of injection	Milligram	Per cent of injection
	grams					
30	101	Normal	7.0	Average (82.8)	0.0120	Average (71.3)
32	136		12.0		0.0187	
33	120		12.5		0.0174	
34	148		13.0		0.0195	
35	133		12.5		0.0202	
32	132		9.3		0.0175	
33	118		8.3		0.0148	
34	138		11.9		0.0183	
35	129		8.9		0.0168	
28	162	Adrenalectomized, no extract	4.5	Average (35.4)	0.0148	Average (67.0)
29	135		3.8		0.0160	
36	123		5.3		0.0156	
37	109		4.6		0.0178	
38	133		2.5		0.0200	
39	120		6.6		0.0200	
36	123	Adrenalectomized, extract-treated	11.0	Average (77.5)	0.0185	Average (75.0)
37	114		8.5		0.0171	
38	134		10.1		0.0208	
39	124		8.8		0.0181	

* Each rat given 0.020 mgm. phenolsulphonphthalein in 10 cc. 0.65 per cent sodium chloride per 100 grams of body weight.

rate of excretion in the dog to be 1.5 gram urea per gram kidney per 24 hours. Since our untreated adrenalectomized rats excreted very little water when injected with large amounts of fluid of low urea concentration, it seemed apparent that small quantities of urea would be as toxic to the adrenalless animal as very large amounts of urea were to the normal rat. This was found to be the case.

Dye excretion. The injection of phenolsulphonphthalein in adrenalec-

tomized and in normal rats resulted in an equally efficient elimination of the dye in both conditions (table 2). Although the normal animals voided much more urine than the operated rats, the excretion of the dye was about 70 per cent in the first 3 hours in each case. It is possible that the dye was eliminated even more rapidly than indicated, but the small amount of urine excreted in the first hour or two made the early determination of the substance impracticable. No detectable amounts of phenolsulphonphthalein were eliminated after the first three hours. The administration of extract along with phenolsulphonphthalein increased the urine output, but did not augment the excretion of the dye.

TABLE 3

Water content of tissues and blood of normal and adrenalectomized (untreated) rats

RAT NUMBER	WATER CONTENT			Blood urea
	Liver	Body tissues*	Blood	
	(A) Normal			
	per cent	*per cent*	*per cent*	*mgm. per cent*
40	70.7	68.1	82.0	4.5
41	70.0	68.3	83.5	4.5
42	71.0	67.7	82.5	4.9
85	71.5	69.4	83.1	6.0
86	69.7	63.9	84.2	6.4
87	71.1	69.3	84.2	6.2
	(B) Adrenalectomized, showing symptoms			
13	77.2	70.5	81.9	
18	72.4	69.9	80.7	68.0
19	76.3	70.1	80.7	
43	74.8	69.5	81.3	28.3
92	74.2	69.1	81.0	37.0

* Less liver and alimentary tract.

Tissue fluids. In the case of rats which were dying of adrenal insufficiency the water content of the liver and the body hash (ground carcass, less liver and gastro-intestinal tract) was considerably higher than that of the tissues of normal animals (table 3). The blood of the adrenalectomized rats showed, however, a subnormal percentage of water.

Both normal and adrenalectomized animals treated with extract in addition to the large amounts of various solutions employed, excreted practically all the excess fluid in the course of 24 hours. When these were sacrificed at the end of the metabolic period, the liver and body hash were found to have approximately the same water content as normal tissues. In the case of adrenalectomized animals treated similarly but without

extract, very little urine was passed and the injected fluid was almost entirely retained. The body tissues in these cases were found to be considerably hydrated.

When a single injection of 10 cc. of isotonic saline per 100 grams of body weight was given intraperitoneally to rats, and the animals killed five hours afterwards (while the salt solution was still being absorbed), it was found that the liver and body hash of the unoperated series averaged 71.9 and 67.2 per cent of water respectively, while the same tissues of the adrenalectomized series averaged 72.6 and 68.2 per cent (compare with table

TABLE 4

Experiments on normal and adrenalectomized cats showing water balance and other conditions in tissues and blood

CAT NUMBER	WEIGHT	WATER CONTENT							Blood		LIVER GLYCOGEN
		Liver	Muscle	Kidney	Spleen	Brain	Skin	Blood	Sugar	Urea	
		(A) Normal fasting									
	kgm.	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*mgm. per cent*	*mgm. per cent*	*per cent*
11	2.14	66.6	74.2	77.4	76.2	76.9	53.5	86.8	68	8.1	0.769
12	1.98	69.3	74.7	75.5	79.0	78.9	49.6	82.8	66	5.0	0.679
13	1.80	71.7	74.8	77.6	78.3	78.9	53.6	85.2	62	7.2	0.688
14	1.30	71.4	75.8	76.8	78.4	78.6	57.8	85.6	76	7.8	0.664
15	1.80	70.6	75.2	77.5	78.6	78.5	49.3	84.8	78	6.1	0.771
16	1.44	71.2	74.7	76.9	78.3	79.4	56.1	85.8	65	7.9	0.660
Average....		70.1	74.9	77.0	78.1	78.5	53.3	85.2	69	7.0	0.705
		(B) Adrenalectomized, showing symptoms									
17	1.47	74.3	75.9	75.8	79.2	78.8	45.4	80.7	36	21.6	0.277
18	2.36	73.0	74.6	75.4	77.9	78.9	53.3	81.1	51	19.7	0.225
19	1.11	73.4	77.5	76.6	79.1	78.8	60.0	81.0	43	15.8	0.200
20	3.56	74.1	77.5	75.2	78.2	77.9	52.2	77.1	37	36.5	0.198
21	2.24	73.3	76.4	76.7	77.7	76.7	52.6	82.5	58	25.0	0.245
Average....		73.6	76.4	75.9	78.4	78.2	52.7	80.5	45	23.7	0.229

3). The blood of the normal rats contained 84.8 per cent water, while that of the operated animals, whose bodies contained an abnormal concentration of fluid, was nevertheless dehydrated and the water content averaged only 81.2 per cent. Administration of cortical extract to adrenalectomized rats under the same experimental conditions resulted in a condition of water balance very similar to that observed in the normal animals.

In a series of bilaterally adrenalectomized cats showing symptoms of insufficiency, it was observed that the hepatic and muscle tissues were hy-

drated and the blood and kidneys dehydrated, in comparison with the 24-hour fasted control animals (table 4). The spleen, brain and skin were unaffected by adrenal removal as far as water content was concerned. The operated series showed, in addition, a uniformly higher blood urea and lower blood sugar and liver glycogen concentration than the unoperated animals. It would seem likely that the dehydrated kidney in the case of adrenalectomized cats was due rather to a relative lack of urine in the tubules and renal pelvis than to actual fluid deprivation in the renal tissues themselves.

Discussion. Marshall and Davis observed that their cats had a tendency towards diminished phenolsulphonphthalein and nitrogen excretion after adrenalectomy. As a result of these and other experiments, they postulated the secretion of some substance by the adrenals which was necessary for the maintenance of normal kidney function. That corticoadrenal extract contains such a substance—or a hormone—is indicated by the results of the work contained herein. Whether the action of cortical extract is directly on the kidneys or indirectly through some other mechanism is a moot point.

The fact that adrenalectomized rats excrete less water than normal rats, and that this deficiency is tremendously magnified by the injection of large amounts of fluid, is suggestive that the kidney itself is limited in its excretion of water. If the elimination of chlorides and urea be expressed in milligrams per cubic centimeter of urine in place of the total amount excreted per 24 hours, there does not appear to be any great difference in the chloride or urea excretion in normal or adrenalectomized rats given large quantities of various solutions (table 5). In other words, the composition of the urine of normal and adrenalectomized rats under this régime is quite similar; the notable difference is in the amount of fluid eliminated.

Many observers have shown that in untreated adrenalectomized animals there is a reduction in blood chlorides (Lucas, 1926; Swingle, 1928), and it appears from the present experiments that the kidney of the uninjected, untreated adrenalectomized rat is able to excrete chlorides rather efficiently, though its ability to excrete water is impaired. However, when the organism is suffering from adrenal insufficiency, this apparent inability of the kidney to excrete water may in fact be indicative of a fairly "normal" renal function in the absence of the adrenal glands. According to Marshall (1926), one of the functions of the kidney is "to excrete chlorides and water in large amounts when present in excess, but to markedly decrease their elimination when the body lacks [or needs] them."

We have shown in previous papers (Britton and Silvette, 1931, 1932) that after adrenalectomy the blood sugar and hepatic and muscle glycogen are greatly reduced. Many observers have pointed to the increase in blood urea after the operation (see Britton, 1930); and in view of the

ready diffusibility of urea it may be assumed that there is a high urea content in the tissues as well as in the blood. In addition, we have demonstrated that the liver and the skeletal muscle of adrenalectomized animals are definitely hydrated in comparison with normal tissues.

Since muscle and liver in the rat constitute somewhat more than half the body weight of the animal, considerable amounts of fluid are represented by the increased water content of these tissues. These observa-

TABLE 5

Table showing average urinary output in rats under different experimental conditions

NUMBER OF RATS IN SERIES	EXPERIMENTAL CONDITIONS*	INJECTED MATERIAL	24-HOUR URINARY EXCRETION					
			Volume		Chloride		Urea	
			Per 100 grams rat	Total	Per cubic centimeter	Total	Per cubic centimeter	Total
			cc.	*cc.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
6	Normal, water *ad lib.*	None	4.5	5.9	2.4	13.9	1.5	9.0
8	Adrenalectomized, water *ad lib.*	None	4.1	4.4	6.1	26.6	1.9	8.5
2	Normal	Water	17.4	23.3	0.5	11.2	0.5	11.1
4	Adrenalectomized	Water	3.9	5.1	1.1	5.1	1.3	6.2
4	Adrenalectomized, extract-treated	Water	17.0	20.4	1.0	19.3	0.7	12.8
4	Normal	NaCl	20.3	27.8	5.4	149.0	0.5	12.8
4	Adrenalectomized	NaCl	8.2	8.4	5.6	47.0	1.0	8.1
4	Adrenalectomized, extract-treated	NaCl	18.4	20.0	5.7	114.0	0.6	11.5
5	Normal	Urea	20.2	27.9	0.6	16.6	1.1	30.0
4	Adrenalectomized	Urea	1.5	2.0			0.5	0.9
5	Adrenalectomized, extract-treated	Urea	18.7	23.2	0.6	14.2	1.4	32.1

* Ten cubic centimeters fluid per 100 grams of body weight; one injection at 0 hour, second injection at end of 6th hour, experiment terminated at end of 24th hour.

tions indicate that there is some necessity in these tissues for increased fluid, possibly to dilute the end-products of katabolism; or to render toxic materials, which in the absence of the adrenal extract may not easily be eliminated, relatively innocuous. With water being drawn into the tissues and hence out of the blood stream in adrenal insufficiency, the osmotic pressure of the blood would tend to rise. Reduction would appear to be brought about in part by means of a diminution in blood chlorides;

correlatively, these are found to be excreted in abnormal concentration through the kidneys.

The transference of water from blood to tissues may be, as Gradinescu suggested (1913), the result simply of an increased permeability of the blood vessel walls, permitting plasma to escape into the hydrophilic tissues. If this train of events actually takes place, the kidneys in adrenalectomized animals would appear to be functioning with a delicate efficiency, holding back water and permitting the elimination of ions which would tend to disturb normal osmotic relationships.

The high blood urea in adrenalless animals may be referable, as we have suggested, probably not to an increased katabolism, but to the relatively small volume of plasma filtered by the kidneys, with consequent damming up of urea in the blood stream and tissues. Durrant (1928) has shown that the blood pressure of adrenalectomized rats is, on the average, 77 mm., while that of normal animals averages 111 mm. This observation would in part account for the apparent inability of the renal mechanism to excrete nitrogenous end-products from the blood.

The influence of carbohydrates on the viscosity of colloids may, in view of the demonstrated hepatic and muscle glycogen deficiency, account for the imbibition of water by liver and muscle. The administration of cortico-adrenal extract, by increasing the glycogen content to normal levels, would tend to liberate water from these (previously hydrated) tissues, which would in turn dilute the blood, produce diuresis and concomitantly reduce the high level of urea in the blood.

The existence of two hormones in our preparation of cortico-adrenal extracts has been postulated in earlier papers (Britton and Silvette, 1931, 1932; Corey and Britton, 1931). The more important component we considered to be the life-maintaining hormone; the dispensable factor that which induced sexual precocity in immature rats. The establishment of facts in regard to the fluid content of tissues and the output of urine under various conditions, particularly under the influence of adrenal extract, stimulates further consideration regarding the presence of two hormonal principles in the extract. Possibly the hypertrophy and hyperactivity of the sex organs which follow injection of the material (Corey and Britton, 1931) may be related initially to the fluid concentration in the gonadal tissues. Since energy changes are involved, the carbohydrate content of the pertinent organs (see Britton and Silvette, 1932) may furthermore be implicated. Investigation regarding these possibilities is now under way.

SUMMARY

Rats from which both adrenal glands have been removed excrete less urine and more chlorides than normal animals under the same fasting con-

ditions. When different fluids are injected into adrenalectomized animals, their reduced ability to eliminate fluids in comparison with unoperated animals is markedly manifested.

In the case of normal rats and adrenalectomized rats treated with cortico-adrenal extract, the chloride output per 24 hours was approximately the same when distilled water or urea or glucose solutions were injected. Slight differences only appeared in the urea output of normal and operated animals when water alone was given, or when sodium chloride or glucose solutions were administered.

In non-extract-treated adrenalectomized rats injected with various solutions there was a striking deficiency in water excretion. The output of chlorides and urea in milligrams per cubic centimeter of urine did not differ greatly, however, from that observed in normal rats or extract-treated adrenalless animals receiving comparable injections.

Urea given in small quantities (0.1 gram per day) proved fatal to adrenalectomized animals within 24 hours. Normal animals, or operated animals receiving extract, tolerated without difficulty doses up to 0.8 gram per day.

The excretion of phenolsulphonphthalein was found to be approximately the same in normal and adrenalectomized animals, whether extract-treated or not.

In adrenalectomized rats showing symptoms of insufficiency, the liver and the extra-hepatic body tissues were found to have a higher water content than similar tissues of normal animals.

In a series of adrenalectomized cats showing symptoms of insufficiency, the liver and skeletal muscle were found to be more hydrated and the blood and kidneys less hydrated than the similar tissues in normal animals. The water content of the spleen, brain and skin was approximately the same in normal and adrenalectomized cats.

From the evidence adduced it is concluded that the inability of the kidney in adrenalectomized animals to excrete water is due to causes which are extra-renal in origin. The hydrophilic condition of the liver and skeletal muscle may be of particular significance in adrenal insufficiency. The water demands of the hepatic and muscular tissues may be referable to deficiency or toxic conditions (possibly to the profound glycogen reduetions) which supervene on adrenal removal.

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A COMPARISON OF THE CLEARANCES OF VARIOUS URINARY CONSTITUENTS

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The clearances of three substances or classes of substances have been proposed as measuring filtration rates, creatinine, inert sugars and inorganic sulphate. If the first is accepted and if creatinine is consistently concentrated more than any other urinary constituent, no secretion of anything need be assumed; if the second or third is accepted, filtration rates from 10 to 70 per cent as great as with creatinine are calculated and the secretion of creatinine, and at times of other substances, must be assumed. In view of the diversity of opinion as to the ideal no-threshold substance it has seemed to us worth while to compare simultaneously the concentration ratios and clearances of glucose under phlorhizin and of the three normally occurring urinary constituents which have been variously proposed as no-threshold substances, creatinine, inorganic sulphate and inorganic phosphate.

METHODS. Large dogs on an unregulated diet were used in all experiments. From 0.24 to 0.27 gram sodium barbital per kilo by leg vein gave in practically all cases a satisfactory anesthesia; with two or three dogs a small amount of ether was given for the abdominal incision. A short cannula for collecting blood samples was put into a femoral artery and an injecting cannula into the opposite femoral vein. Small short soft rubber catheters (1.2 mm. bore) were put into the ureters and urine collected in slender 50 cc. cylinders graduated in tenths of a cubic centimeter, 1 cc. occupying 9 mm. length. Dead space was too small to introduce any significant error. All urine collection periods were 30 minutes; all blood samples were drawn exactly at the middle of urine periods, centrifuged immediately and the serum removed and placed in the refrigerator; the protein-free filtrates were usually prepared on the day of the experiment. No hematuria was observed and no hemolysis except in the first experiments; all urines were free from protein except in experiment 12 where the dog undoubtedly had nephritis. The procedure finally adopted was to phlorhizinize[1] the dog, operate, collect urine for a 30 minute period, give

[1] In most experiments 0.22 gram of Merck's phlorhizin per kilo, purified according to Deuel and Chambers (1925), dissolved in sodium carbonate solution was given

an intravenous injection lasting 20 minutes of 5 cc. per kilo of a 5 per cent Na_2SO_4, 7 per cent Na_2HPO_4 and 1 per cent creatinine solution brought to pH 7.4 by syrupy H_3PO_4, discarding the urine during the injection and for the next 10 minutes, start the second urine collection 10 minutes after the end of the injection and continue for 2 hours, getting 4 more 30 minute urine collections with a blood sample at the middle of each. Twenty-five cubic centimeters of blood were taken for each sample; this together with the 3 or 4 cc. washed out of the cannula before each collection amounted to less than 150 cc. of blood loss for the experiment, which is usually less than 10 per cent of the blood volume. Blood pressure was not taken in the first nine experiments; at the end of the last four experiments, 10, 11, 12 and 13, the mean blood pressures were 124, 108, 134 and 130 mm. Hg, respectively.

In all the experiments except nos. 3, 4 and 5 serum was used, in these 3 experiments plasma with 0.25 per cent NaF as anticoagulant. We returned to serum after the fifth experiment in order to avoid the disturbing effects of the anticoagulant; the magnitude of these effects is being further investigated. The chemical methods are discussed in the appendix. The findings are given in table 1.

The following are among the conclusions to be drawn from the table.

1. No two of the four materials studied show the same concentration ratio or clearance with any approximation to consistency; in this respect the results are a confirmation and extension of the earlier findings of White (1923) and of Underhill (1923). The finding of Hayman and Johnston (1932) and of Cope (1932) that creatinine clearance is greater than sulphate clearance is confirmed. The finding of Poulsson (1930) that the clearance of glucose under phlorhizin is almost equal to that of creatinine is not confirmed; the statement of Shannon, Jolliffe and Smith (1932) that the clearances of creatinine and of glucose under phlorhizin are the same is not confirmed. Even if one assumed that all of the chromogenic material in the serum is creatinine and did not subtract anything from the values obtained for the post-injection samples, the creatinine clearances would still be higher than the glucose, often 50 per cent higher or more.

2. Creatinine is concentrated more than glucose, sulphate or phosphate. The only exception is in experiment 1, where sulphate was concentrated more than creatinine. On the whole the glucose clearances are nearer to the sulphate than to the creatinine but there are many cases where the glucose and sulphate clearances are far apart. In experiments 8 and 13

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subcutaneously about 1½ hours before beginning the first urine collection and another 0.11 gram per kilo between the first and second urine periods. In some cases minor departures from this procedure were employed but with no demonstrable difference in results.

TABLE 1

A comparison of the clearances of four urinary constituents at various serum levels

EXPERIMENT NUMBER, DATE, SEX AND WEIGHT	URINE VOLUME, V	CREATININE		GLUCOSE		S		P		CLEARANCE = $\frac{U V}{Se}$				$\frac{\text{CREATININE CLEARANCE}}{\text{GLUCOSE CLEARANCE}}$	$\frac{\text{CREATININE CLEARANCE}}{\text{S CLEARANCE}}$	$\frac{\text{CREATININE CLEARANCE}}{\text{P CLEARANCE}}$
		Urine	Serum	Urine	Serum	Urine	Serum	Urine	Serum	Creatinine	Glucose	S	P			
	cc. per minute	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*cc. per minute*	*cc. per minute*	*cc. per minute*	*cc. per minute*			
1		213	1.1			127	3.4	444	2.3							
Sept. 28/32	4.23	142	7.7	1,150	118	389	17.0	172	18.2	78.4	41.5	97.0	40.2	1.89	0.81	1.95
M	3.50	145	4.9	1,810	104	398	10.0	180	10.0	104	60.9	139	63.1	1.71	0.75	1.65
20.5 kilos	2.56	151	4.2	2,610	91.6	387	6.2	181	7.1	92.2	73.0	159	65.3	1.27	0.58	1.41
	1.88	141	3.3	3,670	83.1	362	4.6	178	4.9	80.5	83.1	148	68.4	0.97	0.54	1.18
2	0.173	111	1.0		243	205	3.9	469	5.3			9.1	15.3			
Oct. 12/32	1.11	165	10.1	2,990	203	256	21.1	184	22.0	18.1	16.3	13.4	9.3	1.11	1.35	1.95
M	0.31	164	8.7	3,150	208	228	21.5	149	20.0	5.9	4.7	3.3	2.3	1.26	1.79	2.56
15.7 kilos	0.073	154	8.2	3,200	216	192	20.8	121	20.5	1.4	1.1	0.7	0.4	1.3	2	3.5
	0.023	156	7.9	3,060	224	264	20.1	135	22.0	0.5	0.3	0.3	0.14	1.7	1.7	3.6
3	0.613	141	1.3		91.6	24.6	2.9	15.7	4.0			5.2	2.4			
Oct. 24/32	3.37	243	8.1	1,580	85.6	339	17.4	260	18.5	101	62.3	65.7	52.4	1.62	1.54	1.93
M	2.52	216	4.5	2,260	77.8	288	10.3	243	13.0	122	73.3	70.5	47.1	1.66	1.73	2.59
25.5 kilos	1.97	190	3.2	2,900	77.9	266	6.4	209	9.7	117	73.1	82.0	42.4	1.60	1.43	2.76
4	1.01	33.3	0.74		109	25.5	5.2	51.4	7.0			5.0	7.4			
Oct. 28/32	3.35	163	5.1	2,460	108	262	13.5	174	14.7	107	76.3	65.0	39.6	1.40	1.65	2.70
F	2.26	143	2.9	3,540	98.5	198	9.5	158	11.4	111	81.1	47.1	31.3	1.37	2.37	3.55
14.2 kilos	1.63	142	2.2	4,710	87.5	171	8.5	154	9.0	105	87.6	51.6	27.9	1.20	2.04	3.76
	1.36	146	1.4	5,450	80.3	174	6.4	168	8.1	141	92.4	51.4	28.2	1.53	2.76	5.00
5	0.63	60	1.2		129	42.2	4.1	<1	6.0			6.5	<0.1			
Nov. 1/32	2.21	187	6.7	1,780	109	335	16.0	191	17.7	61.6	36.1	46.4	23.8	1.71	1.33	2.59
M	1.57	177	4.3	2,550	95.2	292	12.8	189	13.2	64.8	42.1	35.8	22.5	1.54	1.81	2.88
14.0 kilos	1.28	156	3.2	2,800	87.2	263	9.4	179	11.5	62.5	41.2	35.8	19.9	1.52	1.75	3.14
	1.11	158	2.3	2,960	72.2	247	8.2	189	9.8	76.4	45.6	33.4	21.4	1.67	2.28	3.57
6	0.59	80.8	0.85	11,500	123			25.4	3.3		55.1		4.5			
Nov. 4/32	2.36	163	6.4	2,090	121			208	12.2	60.2	40.9		40.4	1.47		1.49
F	1.21	204	3.5	4,220	98.4			241	7.5	70.6	51.9		39.8	1.37		1.77
13.2 kilos	0.90	196	2.9	4,960	87.8			231	6.5	60.8	50.8		32.0	1.19		1.90
	0.71	205	2.2	5,580	82.1			248	6.2	66.0	48.2		28.4	1.37		2.32
7	0.407	78.4	1.2	10,750	160	24.5	2.7	24.5	6.0		27.4	3.7	1.67			
Nov. 18/32	1.76	189	9.8	2,360	179	336	26.6	249	23.0	34.0	23.2	22.2	19.0	1.46	1.53	1.79
M	1.25	164	7.6	3,040	166	357	20.5	193	18.6	27.0	22.9	21.8	13.0	1.17	1.24	2.08
17.7 kilos	0.95	163	6.7	3,560	171	320	15.6	169	15.8	23.1	19.8	19.6	9.8	1.17	1.17	2.09
	0.78	164	5.6	4,000	154	307	12.6	164	13.2	22.8	20.7	19.0	8.8	1.20	1.20	2.59
8	0.787	104	1.4	8,640	34	21.1	2.7	285	6.0		200	6.1	33.4			
Nov. 23/32	3.10	301	3.2	1,720	26	406	12.1	339	11.0	292	205	104	95.4	1.43	2.81	3.06
F	1.35	359	1.8	3,560	24	348	8.2	483	9.8	270	200	57.4	66.6	1.35	4.71	4.05
28.0 kilos	0.96	361	1.1	4,600	22	237	7.3	493	7.8	315	201	31.2	60.7	1.57	10.1	5.19
	0.73	361	0.9	5,200	20	237	6.3	470	7.8	310	208	18.2	49.0	1.49	17.0	6.33

M	1.25	164	7.6	3,040	166	357	20.5	193	18.6	27.0	22.9	21.8	13.0	1.17	1.24	2.08
17.7 kilos	0.95	163	6.7	3,560	171	320	15.6	193	15.8	33.1	19.8	19.5	9.8	1.12	1.23	2.60
8	0.787	104	1.4	8,640	34	21.1	2.7	255	6.0		200	5.1	33.4			
Nov. 23/32	3.10	301	3.2	1,720	26	406	12.1	339	11.0	292	205	104	95.4	1.43	2.81	3.66
F	1.35	359	1.8	3,560	24	348	8.2	483	9.8	270	200	57.4	66.6	1.35	4.71	4.05
28.0 kilos	0.96	361	1.1	4,600	22	237	7.3	493	7.8	315	201	31.2	60.7	1.57	10.1	5.19
	0.803	347	0.9	5,190	20	154	6.8	476	7.8	310	208	18.2	49.0	1.49	17.0	6.33
9	0.340	125	0.93	15,270	102	96.7	3.3	91.2	4.8		51.0	10.0	6.5			
Nov. 29/32	1.56	271	7.8	2,600	Lost	469	17.0	257	19.2	54.1		43.0	20.9		1.26	2.58
F	0.947	259	4.7	4,200	132	452	12.4	242	14.4	53.1	30.1	34.5	15.9	1.76	1.54	3.34
15.0 kilos	0.720	271	3.8	4,880	145	448	10.8	203	10.9	51.4	24.3	29.9	13.4	2.12	1.72	3.84
	0.513	241	3.2	6,190	147	362	9.6	190	10.1	38.6	21.6	19.4	8.7	1.79	1.99	4.44
10	0.520	89.3	1.0	12,930	129	13.4	3.4	12.2	6.1		52.5	2.1	1.0			
Dec. 7/32	2.90	186	7.0	1,915	107	260	17.1	207	18.9	77.1	51.9	44.4	31.6	1.49	1.74	2.44
M	1.91	182	3.9	3,140	100	258	11.7	214	15.7	89.2	60.0	42.2	26.0	1.49	2.12	3.43
19.0 kilos	1.48	173	3.0	4,220	107	208	9.2	236	11.9	85.4	58.4	33.5	29.3	1.46	2.55	2.91
	1.22	169	2.8	5,030	103	177	7.9	228	11.1	73.7	59.5	27.3	25.0	1.24	2.70	2.95
11	0.577	88.1	1.0	10,400	101	58.4	3.7	52.6	3.1		59.4	9.4	9.8			
Dec. 12/32	1.60	324	7.7	3,420	119	541	16.9	20.8	2.9	67.2	45.9	51.6	11.5	1.47	1.30	5.84
M	0.803	305	5.1	4,890	125	432	14.8	14.6	3.5	48.0	31.5	23.5	3.4	1.53	2.04	14.1
19.5 kilos	0.763	258	4.3	5,550	126	361	11.5	13.0	4.5	45.8	33.6	24.2	2.3	1.36	1.89	19.9
	0.537	267	3.7	6,650	115	341	9.5	18.9	4.8	38.7	31.1	19.3	2.1	1.25	2.01	18.4
12	0.600	73	6.0	2,190	283	8.8	5.3	10.1	7.1		4.6	0.96	0.84			
Dec. 15/32	0.723	151	14.0	1,825	288	11.7	4.0	68.5	19.8	7.8	4.6	2.1	2.5	1.70	3.72	3.12
M	0.703	137	10.1	1,920	271	12.2	4.6	59.3	18.1	9.5	5.0	1.9	2.3	1.90	5.0	4.13
21.2 kilos	0.730	126	10.1	1,900	258	13.0	4.6	56.6	17.1	9.1	5.4	2.0	2.5	1.69	4.55	3.64
	0.730	122	9.8	1,855	252	14.0	4.5	52.6	16.8	9.1	5.4	2.3	2.3	1.69	4.0	4.0
13	0.353	79	1.5	8,670	203	13.8	3.9	<1	3.4		15.1	1.2	<0.1			
Dec. 23/32	0.477	488	8.3	7,830	188	20.8	3.5	<1	4.6	28.1	19.9	2.7	<0.1	1.41	10.4	>300
M	0.450	416	6.0	7,940	174	24.8	5.7	<1	4.8	31.2	20.6	1.9	<0.1	1.52	16.5	>300
16.0 kilos	0.463	370	5.3	7,790	164	26.7	4.3	<1	5.6	32.3	22.0	2.9	<0.1	1.47	11.1	>300
	0.500	336	4.8	7,500	157	32.9	4.3	<1	6.2	35.0	23.9	3.8	<0.1	1.47	9.2	>300

In experiment 11 the phosphate was omitted from the injected solution, in experiment 12 sulphate and in experiment 13 both phosphate and sulphate. Part of urine 1 in experiment 1, all of urine 5 in experiment 3 and the second serum glucose in experiment 9 were lost. Serum and urine glucose in the first period of experiment 1 and urine glucose in the first period of experiments 2, 3, 4 and 5 were not determined. The sulphate figures in experiment 6 have been omitted because the acetone benzidine solution was beginning to give high and inconstant blanks. Serum glucose determinations in experiments 3 to 11, inclusive, were made on 1 to 3 iron filtrates. We now know that glucose values with 1 to 3 dilutions are always somewhat higher than with 1 to 10 or 1 to 12 dilutions and the difference is not constant for various samples. After this was recognized we made determinations on 9 bloods in the last two experiments with both 1 to 3 and 1 to 12 dilutions. The 1 to 3 dilutions in these nine comparisons gave values 1.0, 5.7, 6.7, 8.5, 5.0, 1.0, 7.0, 3.5 and 2.5 per cent higher than the 1 to 12, an average of 4.5 per cent. The corrected figures for experiments 3 to 11, inclusive, as given in the table, have therefore been obtained by multiplying the observed figures obtained on 1 to 3 filtrates by 0.955. The serum glucose figures given for experiments 1, 2, 12 and 13 were actually determined at 1 to 12 dilution.

In our opinion one will be nearest the truth by assuming that none of the pre-formed chromogenic material in blood or serum filtrates is creatinine. We have therefore made a "creatinine" determination on normal serum in the first period and subtracted this figure from the figures obtained after creatinine administration. The differences, representing the minimum amounts of creatinine known to be present, are given in the table as the serum creatinine values for the last four periods in all experiments. On this basis one cannot calculate creatinine clearances for the first periods.

the glucose clearances are many times the sulphate while in experiment 1 the sulphate clearances are greater.

3. The concentration ratio of phosphate may be considerably less than unity. This has been reported before by White (1923) and by Havard and Reay (1926). In both these reports this was seen only with rather marked diuresis. In our work it has been seen with 6 urines (first period of expt. 5 and all of expt. 13) with little or no increase in normal rate of urine flow. We have never seen it after the plasma phosphate level has been raised by injection of phosphate. Since we now know (Walker, Ellinwood and Reisinger, 1932; White, 1932) that the concentration of inorganic phosphate in the glomerular fluid is essentially the same as in the plasma, at least in amphibia, a tremendous capacity for reabsorbing phosphate must be postulated. This capacity is not always exhibited; the conditions which bring it into play are not known. Tubular reabsorption of phosphate may or may not behave according to predictions based on rate of passage of fluid down the tubules with corresponding times available for reabsorption.

4. The creatinine and glucose clearances are not related to their plasma levels and tend to remain constant.

5. Sulphate clearances are always greatly increased by raising the plasma sulphate level above the normal. As the plasma sulphate level falls during the four post-injection periods the sulphate clearance falls with rough proportionality. The only exception is dog 2, which behaved atypically in many respects.

6. The phosphate clearance shows no relation at all to the plasma phosphate level.

Discussion. The discussion will be confined to a few of the conclusions to which the data lead. The concept of no-threshold substances, as applied to normal urine or plasma constituents, is weakened by the finding that no two of them have the same concentration ratio. So long as no two have the same ratio the argument that some one substance *may* be no-threshold remains a pure assumption and the question of what substance to use as a measure of glomerular filtration remains problematical. Rehberg's (1926) choice of creatinine because it has the highest concentration ratio, together with the modification of passive back-diffusion of "low-threshold" substances, offers an escape from the untenable position to which the original no-threshold concept leads. Rehberg's view will explain at least qualitatively the findings in this paper provided one makes enough assumptions as to the permeability of the tubular cells to various substances. For instance, we must assume in experiment 1 that in the first period the tubules are much more permeable to sulphate (concentration ratio 37) than to phosphate (concentration ratio 191) while in the other four periods the relation is reversed. They are more permeable to glucose than to

creatinine in the second period; this relation changes until in the fifth period they are slightly more permeable to creatinine. In the first period they are more permeable to sulphate than to phosphate but in all other periods they are less permeable to sulphate than to any other substance. In the second experiment they are more permeable to sulphate than to phosphate in the first period, in the other periods less permeable. Of 59 periods available for comparison the tubules are definitely more permeable to sulphate than to phosphate in 6, approximately the same (within 20 per cent) in 12, and definitely less permeable to sulphate (often a many-fold difference) in 41. A comparison of glucose and sulphate shows the same varying and interchangeable relation of their capacities to diffuse through the tubule walls.

If we confine our attention to one substance in one experiment Rehberg's view gives a qualitatively satisfactory explanation of its behavior. As an example we may consider sulphate in experiment 8. According to Rehberg a lower clearance of any substance in one period than in another may be due to any one or all of the three following factors, lower glomerular filtration, greater active reabsorption or more complete back-diffusion. The lower sulphate clearance in period 5 than in period 2 cannot be due (following Rehberg's view) to the first of these, as is shown by the constancy of creatinine (and of glucose) clearances, nor to the second. The third possibility, passive back-diffusion, is more satisfactory. Concentration of sulphate in the reabsorbed fluid is 6.06 mgm. S per 100 cc. in the second period and 5.0 mgm. S per 100 cc. in the fifth, the absolute amounts reabsorbed 12.21 and 10.31 mgm. S, respectively. We thus have 84 per cent as much sulphate diffusing back in the fifth period as in the second, although the diffusion gradient of sulphate between urine and plasma is only 37 per cent as great in the fifth period. However, since the urine flow is slower in the fifth period a more complete back-diffusion is expected.

If we attempt a more nearly quantitative comparison, for instance of the first and second periods, the results may not be quite satisfactory but cannot be considered as absolutely irreconcilable with the theory. Let us take glucose clearances as being proportional to glomerular filtration, since no creatinine clearance figures can be given for the first period. This should be permissible since the glucose clearances in the other periods are a constant fraction of the creatinine clearances; the conclusions would not be changed by multiplying all the filtrate figures by 1.5. We calculate that 5.23 mgm. S diffuses back in the first period and 12.21 in the second, or 2.34 times as much back-diffusion in the second, while the sulphate diffusion gradient is 21.4 times as great. The urine flow in the second period is 3.94 times as fast as in the first. The time available for diffusion in period 1 cannot be more than $\frac{R_2}{R_1}$ times as great as in period 2, where R_1

and R_2 are respective rates of urine flow. It must be something less than this because the rate of passage down the first part of the tubule, before water reabsorption has proceeded to any significant extent, is the same in the two periods. So far as tubular surface area is concerned a greater opportunity for diffusion should exist in the second period. Furthermore, the passage of a given small amount of sulphate across the cells would lower the diffusion gradient less in the second period than in the first. We thus conclude that the amount of sulphate diffusing back in the second period, if the process is a simple diffusion, should be not less than $\frac{21.4}{3.94}$ or 5.44 times as much as in the first; it actually is 2.34 times as much. The fact that only 43 per cent as much back-diffusion of sulphate takes place in the second period as is predicted on the Rehberg basis cannot, of course, be considered as a definite refutation of the theory; the calculation is presented to show that even Rehberg's modification does not necessarily remove all the difficulties of the pure filtration-reabsorption viewpoint.[2]

In most of our experiments the amount of glomerular filtrate demanded by the creatinine clearance has not been unreasonable. Determinations of renal blood flow have given widely varying results with different investigators. The highest blood flow found by Mayrs and Watt (1922) in rabbits was 4 cc. per minute for the two kidneys per 100 grams body weight, the average about half of this. If we apply their highest figure to our dogs we find with dog 4 a renal blood flow of 568 cc. per minute. Assuming 60 per cent plasma we get 341 cc. plasma per minute. The creatinine clearance in the fifth period was 141 cc. per minute. This requires that 41 per cent of the plasma be filtered off. Similarly, with dog 8 we must have 47 per cent of the plasma filtered off. We do not insist that these figures mean that a sufficient amount of filtrate is impossible. These are unusual performances and it is possible that these dogs had higher ratios of kidney to body weights and higher rates of renal blood flow than even the maximum in Mayrs and Watt's rabbits and that the plasma volume was more than 60 per cent of the blood volume.

An irrefutable decision between pure filtration-reabsorption and tubular secretion is not justified on the basis of these experiments. The fact that

[2] The limitations of such a calculation are appreciated. We believe, however, that the expression

$$\frac{\text{ratio of diffusion gradients}}{\text{ratio of rates of urine flow}}$$

justly represents the minimum expected ratio of amounts diffusing back; this in spite of another factor, suggested by Dr. A. P. Briggs, that although the surface area is increased in a distended tubule as the first power of the diameter the cross-sectional area of the contained fluid column is increased as the second power. The extent to which this would retard diffusion depends upon the degree of turbulence of flow; this cannot be evaluated but is probably small.

the urinary constituents cannot be arranged in a constant order, even in a single experiment, with respect to their back-diffusibility demands that the process by which the tubule cells resist the entrance of these constituents be not such a passive one as might be concluded from earlier publications. Unanimity of opinion on the question whether such a varying and interchangeable relationship of tubular permeabilities to various substances is more acceptable than tubular secretion can hardly be expected at present.

Our work has so far failed to show that any two urinary constituents consistently show the same concentration ratio. Such a demonstration, which has been reported for several sugars in normal dogs and for sugars and creatinine in phlorhizinized dogs by Shannon, Jolliffe and Smith, appears to us essential to the pure filtration-reabsorption theory; without it all calculations are based on mere guesses. We have begun comparisons of the same type as those reported from Smith's laboratory, upon which we hope to report in the near future.

SUMMARY

1. The concentration ratios and clearances of creatinine, glucose, inorganic sulphate and inorganic phosphate in phlorhizinized dogs have been compared.

2. No two of these substances show the same concentration ratio with any approximation to consistency.

3. Except in one experiment creatinine is always concentrated more than any of the other three substances.

4. Since creatinine is almost certainly secreted by the tubules at normal plasma creatinine levels it seems improbable that they would fail to secrete any exogenous creatinine.

5. We believe that creatinine clearances with normal blood creatinine levels are not determinable.

6. The concentration ratio of inorganic phosphate may be considerably less than unity even with only slightly increased rates of urine flow.

7. In seven experiments out of ten the concentration ratio of sulphate is greater (usually much greater) with high plasma sulphate levels than with normal; in one (expt. 2) the reverse is true, while in two (expts. 1 and 9) the ratio with higher levels is sometimes greater and sometimes less than with the normal level.

8. In seven experiments out of eleven the concentration ratio of phosphate is greater with high plasma levels than with normal, in two (expts. 1 and 2) the reverse is true and in two (expts. 8 and 9) the ratio with high levels is sometimes greater and sometimes less than with the normal level.

9. The creatinine and glucose clearances are not a function of their plasma levels and are relatively constant.

10. The sulphate clearances are always greater with high plasma sulphate levels than with normal, with a rough proportionality.

11. The phosphate clearances are not related to the plasma phosphate level and are absolutely inconstant.

12. The glomerular filtration rates demanded by the creatinine clearances are sometimes very high but perhaps in no case so high as to be impossible.

13. The back-diffusion concept does not give a satisfactory quantitative explanation of certain findings.

14. The back-diffusion concept offers some qualitative difficulties in that in one and the same animal there must be assumed a varying and interchangeable relationship between the permeabilities of the tubular cells to the various constituents.

15. A final decision on the respective merits of the pure filtration-reabsorption and the tubular secretion views for the mammalian kidney cannot be made at the present time.

APPENDIX

CREATININE. *Urine.* An amount of urine (generally 1 cc.) containing from 0.8 to 2.5 mgm. creatinine is placed in a 50 cc. volumetric flask. One and 2 cc. of a creatinine standard containing 1 mgm. per cc. are transferred at the same time to similar flasks and 10 cc. of saturated picric acid (purified according to Folin and Doisy, 1917) and 0.75 cc. of 10 per cent NaOH added to each flask. After ten minutes they are made up to volume and read. A compensating colorimeter was used but is not necessary for the urine determinations.

Serum. Serum creatinine determinations were carried out on the iron filtrates prepared as described under the section on glucose. As suggested by Steiner, Urban and West, the slight excess of barium in these filtrates was precipitated by a drop of saturated Na_2SO_4 and the precipitate filtered off. The determination was as follows: for normal serums (from the first blood samples in each experiment) 5 or 6 cc. of filtrate were transferred to a test tube. For all serums after the injection of creatinine 3 cc. of filtrate (1 cc. serum) were used. In all cases the volume was made up to 6 cc. A blood standard containing 0.02 mgm. creatinine per cc. was prepared the same day by a 1 to 50 dilution of the urine standard. One, 2 and 4 cc. of this blood standard, made up to 6 cc., furnished standards covering the expected range. Five cubic centimeters of alkaline picrate (made up just before using by adding 1 part of 10 per cent NaOH to 10 parts of saturated picric acid) were added and the colors read in a Hastings compensating colorimeter after 7 to 10 minutes. The results on creatinine recoveries by the method described above are seen in table 2.

GLUCOSE. Serum glucose determinations were carried out by a Shaffer-Hartman reagent on a modification of the iron filtrate described by Steiner, Urban and West (1932); the composition of the copper reagent used is given in Steiner, Urban and West's paper. Since the same filtrate was to be used for both glucose and creatinine determinations and since a filtrate more concentrated than 1 to 12 was considered desirable for creatinine determinations our filtrates were prepared as follows. Four cc. of serum, 6 cc. water and 2 cc. 12 per cent ferric sulphate are measured into a 50 cc. Erlenmeyer flask. After mixing, 2 grams $BaCO_3$ are added, the flask shaken, then stoppered and thoroughly shaken. After centrifugation and filtration about

7 cc. of filtrate are obtained; 2 cc. are used for the glucose determinations. The amount of ferric sulphate per cubic centimeter of serum employed in this precipitation, although considerably less than that needed for whole bloods, gives protein-free filtrates. Glucose values on these filtrates agree within 1 per cent with the values obtained on filtrates similarly prepared but with 3 times this amount of ferric sulphate. Our later finding of a discrepancy between serum glucose values on 1 to 3 and 1 to 12 filtrates is referred to in the legend accompanying the table, together with the method adopted to obtain correct figures.

Urine samples were diluted so that 5 cc. contained approximately 1 mgm. glucose and the same glucose method used as with serum. Non-glucose reducing substances were not removed from the urines; in only one or two urines could they have been more than 5 per cent of the total reducing substances and in most cases considerably less than this.

SULPHATE. Inorganic sulphates were determined by the method of Power and Wakefield (1931); the details of this method were kindly communicated to us by the authors. A sample of C. P. trichloracetic redistilled *in vacuo* was used in the first experiments. We then found that Eastman's trichloracetic acid is sulphate-free, i.e., 0.3 gram trichloracetic dissolved in 1 cc. saturated $BaCl_2$ gives no cloud. In our experience no other brand of trichloracetic has been of sufficiently low sulphate content to permit its use without distillation.

TABLE 2

BEEF SERUM "CREATININE"	ADDED CREATININE	RECOVERED CREATININE	THEORETICAL CREATININE
mgm. per 100 cc.	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*
1.6	10	11.7	11.6
1.6	20	21.5	21.6
1.4	5	6.3	6.4
1.4	10	11.1	11.4

After a preliminary period of work with blanks and controls we adopted the aqueous benzidine hydrochloride solution of Stadie and Ross (1925) instead of the acetone benzidine solution. The aqueous solution must be filtered each time used. Recoveries from known pure sulphate solutions on samples ranging from 0.02 to 0.2 mgm. S were within 5 per cent and usually within 2 per cent of the theoretical; the same is true of known amounts of sulphate, from 4 to 10 mgm. S per 100 cc., added to serum. It was found necessary to run a blank on a sulphate-free serum filtrate and subtract this from the original figure; the blank is usually about 10 per cent of a normal serum sulphate but varies considerably. The acetone benzidine solution was used in the first 6 experiments; the aqueous benzidine solution in the last 7.

Urine sulphates were determined by the same method. Dilutions were made so as to have approximately 0.2 mgm. S in the sample analyzed. Blanks on sulphate-free samples of the first urine collection were carried out and subtracted from the original figures. The blanks varied considerably but were negligible as compared with the sulphate content of the urine samples after sulphate injection and were therefore carried out only on the first samples of each experiment. Duplicates were determined frequently but not invariably on both serums and urines; they were usually within 1 per cent of the mean and on only one determination were they more than 3 per cent from the mean. We have found it advantageous to carry out the titrations by artificial light in order to prevent re-oxidation of the chromic acid.

PHOSPHATE. Serum phosphates were determined on a portion of the trichloracetic filtrate used for sulphate, using the method of Benedict and Theis (1924). Urine phosphates were determined by a modification (Briggs, 1932) of the Briggs method (1922). In the first experiment simultaneous determinations of urine phosphates by the Benedict-Theis and Briggs methods gave for the five urine samples 445 and 443, 169 and 174, 180 and 179, 180 and 182, 180 and 176 mgm. P per 100 cc., respectively, indicating the absence of acid-hydrolyzable organic phosphates in dog urine.

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THE CIRCULATORY EFFECTS OF ACUTE EXPERIMENTAL HYPERVOLEMIA

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Polycythemic hypervolemia is a condition in which the total blood volume as well as the erythrocytes are increased (Rowntree, Brown and Roth, 1929). *A priori* it might be anticipated that the hemodynamic effects would be similar to those produced through increased venous return plus a greater viscosity of blood. In other words, the venous pressure would be expected to rise, the diastolic size of the ventricles to increase, the systolic discharge to augment and the arterial pressures to become higher.

The earlier experiments of Worm Müller (1873), of Johannsen and R. Tigerstedt (1889) and the more recent investigations of C. Tigerstedt (1908) appeared to support these conclusions. Considerable evidence, both clinical and experimental, has however been presented which seems to show that these consequences do *not* occur. Thus, a number of investigators have reported that the cardiac output is not increased in clinical cases of polycythemia vera (Liljestrand and Stenström, 1925–26; Ernst, 1930), and others report that cardiac hypertrophy is absent unless arterial changes are also present.

Hess (1909) was able to maintain a state of polycythemia for several months in rabbits by repeated injections of blood. He found no evidence of cardiac hypertrophy at the end of this period. Meek and Eyster (1922) were able to increase the blood volumes by 33 to 100 per cent by means of gum acacia or blood infusions. However, with one exception, no evidence of increased diastolic size was found in x-ray films. Supplementary studies led to the conclusion that the infused blood or acacia solution is largely accommodated in dilated capillaries and venules, evidence for which was obtained by study of the skin vessels. But Beck and Holman (1925) who carried out similar experiments noted some increase in heart size of dogs after infusion of whole blood, also an acceleration of the heart.

It is obvious that our knowledge of the cardiovascular changes produced by polycythemic hypervolemia is in some respects fragmentary and in others contradictory. In addition, it revives the questions: 1. Can the dynamic reactions established upon the exposed heart, freed from the pericardium be applied to the heart within the closed chest? 2. Are

the blood reservoirs sufficiently capacious and adaptable under normal conditions to withdraw enough blood from circulation so that the volume returned to the heart remains normal? The circulatory changes following experimental polycythemic hypervolemia were therefore reinvestigated by methods not hitherto applied to its study.

PROCEDURE. Artificial polycythemic hypervolemia was produced by injecting over a period of an hour or somewhat less, a considerable quantity of concentrated heparinized blood into dogs anesthetized with morphine and sodium barbital. The blood used was drawn from a second large dog into heparin solution (1.5 mgm. per 5 cc. of blood). This blood was allowed to settle overnight in an icebox and in the morning enough plasma could usually be decanted to reduce the volume of blood to 60 per cent of the original. This method has the advantage over centrifugation that the corpuscles are not agitated and hemolysis does not occur.

This sedimented blood, warmed to the temperature of the animal, was injected at a controlled rate into the femoral vein of the experimental animal by means of the apparatus diagrammatically shown in figure 1. It consists of a quart thermos bottle, *A*, connected to a source of pressure for displacing the blood. A mercury safety valve, *B*, kept the blood under constant pressure. The outflow tube was connected to a dropping bulb, *C*, by which the rate of flow could be gauged visually. The volumes injected could be determined by noting the descent of a fine glass tube attached to a float resting on the blood. This was surrounded by a calibrated glass tube, *D*, closed at the top. The rate of injection in different experiments ranged from 0.45 cc. to 1.65 cc. per kilo per minute and the total amounts of blood from 250 to 500 cc. Temperature readings were made by a thermometer, *E*, inserted into the thermos bottle. This was kept at approximately 40°C. The periods of injections varied from 30 to 60 minutes but observations were continued for an additional 2 to 4 hours. The degrees of polycythemia and roughly also of the hypervolemia were estimated by making repeated red cell counts of the arterial blood.

The changes in circulatory dynamics of naturally breathing dogs were followed by recording the pressure pulses from the innominate artery by means of Wiggers' universal optical manometer calibrated under static conditions. From such records it was possible to determine changes in heart rate and in the successive phases of systole. From the numerical systolic and diastolic pressures, interpreted in the light of contour changes during the ejection phase, deviations in the mechanism of ejection were inferred. Studies of the diastolic gradient in relation to absolute pressure levels allowed an estimation of changes in total peripheral resistance.

In addition to these optical records, consecutive changes in the effective venous pressure, in respiratory rate and volume, in spleen volume and in

urinary flow were recorded simultaneously on a smoked drum by suitable apparatus.

In order to substantiate conclusions reached on the basis of such experiments, the chests of some dogs was opened under artificial respiration and tracings of left ventricular pressures were taken synchronously with those from the aorta. In these experiments, the pericardium was left intact except for a small slit for insertion of the manometer. The pressure curves from the left ventricle enabled a direct determination of changes in initial tension and of the gradients of contraction and relaxation, thus allowing a more detailed study of primary and secondary factors responsible for the cardiac reactions.

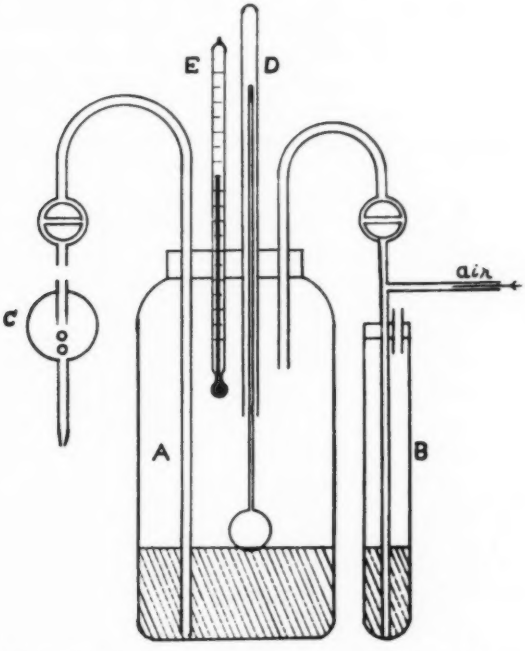


Fig. 1. Blood infusion apparatus. Description in text

RESULTS. During the infusion of concentrated blood, the red cell counts of arterial blood increased steadily; not infrequently, a further increase occurred for some time after cessation of infusion. The counts always remained far above normal for the additional 2 to 4 hours that

TABLE 1

EXPERIMENT	NORMAL	END OF INFUSION	1 HOUR LATER	2 HOURS LATER	3 HOURS LATER
	$\times 10^6$	$\times 10^6$	$\times 10^6$	$\times 10^6$	$\times 10^6$
AA56	7.0	7.8	8.4	8.05	
AA57	6.0	10.1	11.2	11.2	9.0
AA58	5.1	8.0	9.0	9.3	
AA61	5.7	7.4	7.7	8.1	
AA62	5.1	8.5	8.5	8.7	8.5
AA64	6.3	9.1	9.5	9.0	9.0
AA66	5.6	7.1	7.6	7.6	7.6
AA73	4.5	8.6	8.5	8.4	

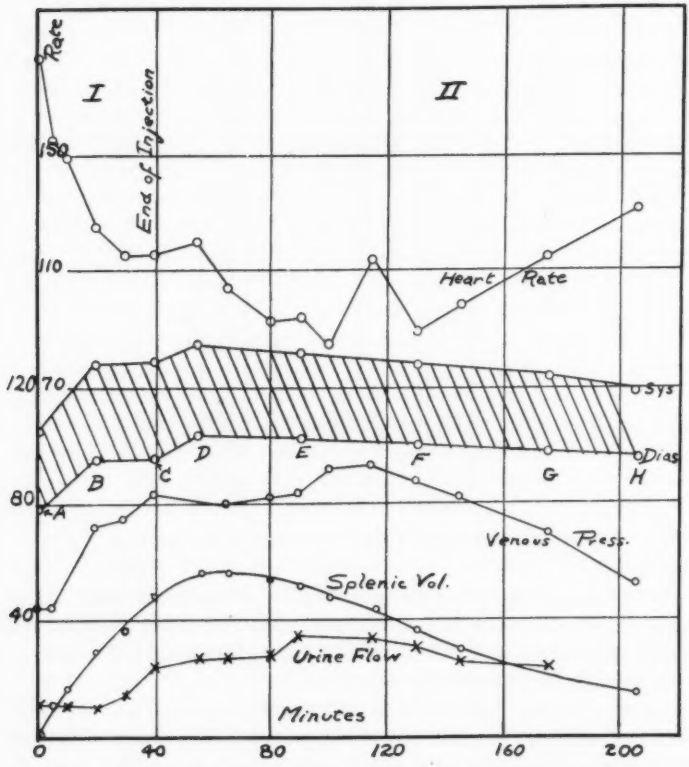


Fig. 2. Plot of successive changes in heart rate, blood pressure, effective venous pressure, splenic volume and urine flow. Abscissae time in minutes. Discussion in text (expt. AA71).

observations were continued. This is shown by the data of table 1 in which the counts are expressed in round numbers.

The trend of dynamic events is best illustrated by a graph of a representative experiment shown as in figure 2. For descriptive purposes, the chart is divided into two parts (I, II), corresponding roughly to stages of rapidly increasing and sustained polycythemia.

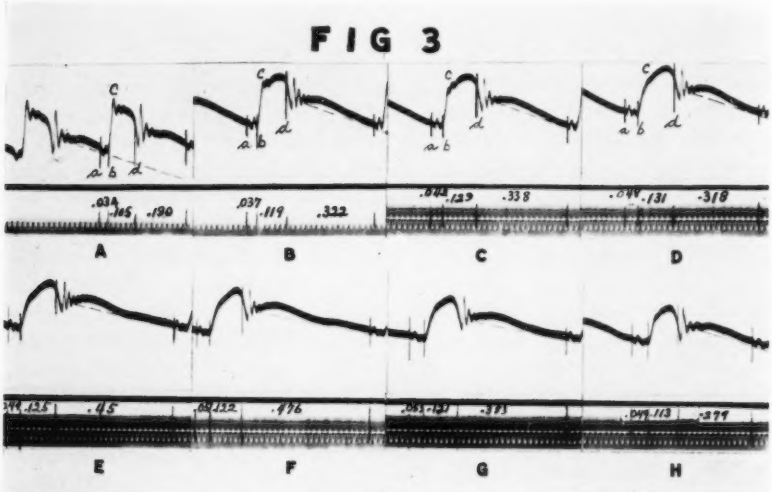
During a period of 40 minutes (I) a total volume of 450 cc. of concentrated blood was injected at a rate of 9 cc. per minute. The graph shows that the effective venous pressure (i.e., the difference between thoracic and intra-auricular pressure at the end of expirations) increases rapidly and the elevation continues after completion of the infusion. The most constant change is a prompt decrease in heart rate which continues after termination of the infusion. The spleen begins to expand within five minutes after starting the injection and becomes enormously distended during the course of infusion. The urinary flow increases markedly, in this way decreasing the total plasma volume but augmenting the corpuscular concentration and blood viscosity at the same time. Despite the pronounced cardiac slowing, blood pressures increase significantly, systolic more than diastolic. In other words, the pulse pressure becomes larger.

After completion of the blood infusion (II), the effective venous pressure for a time remains at the level reached at the end of infusion, but in the experiment plotted it rises to a second peak, *E-F*. It then gradually declines but at the conclusion of observations it is still definitely above the starting level. The slowing of the heart continues until *F*, after which a gradual acceleration takes place. The spleen volume begins to decrease shortly after completion of the infusion; it continues to decrease steadily until the original volume is practically regained at the end of the observations. Diuresis persists throughout the experiment; indeed the urine flow increases somewhat until *E* and then gradually diminishes. An effort to calculate roughly the fate of 450 cc. of blood injected during the course of the experiment showed that the spleen volume increased 47 cc. and the urinary output 280 cc., thus accounting for somewhat more than one-half of the fluid injected.

At various times during the experiment arterial pressure pulses were recorded optically. A few illustrative curves, recorded at times denoted by letters *A*, *B*, *C*, *D*, etc., in figure 2 are reproduced in segments of figure 3, similarly labeled for cross-reference. An examination of these original records shows that systolic and diastolic pressures reach their maximum elevation at *D*, after which systolic and diastolic pressures both steadily decline and pulse pressure similarly decreases.

The progressive changes in duration of the isometric contraction and ejection phases marked on the records are significant. During the period of infusion (segments A-C) the isometric contraction phase (*a-b*), as well

as the ejection phase (*b-d*) increase steadily. Comparisons with calculated values for theoretical systoles (cf. Wiggers, 1923) indicate that the prolongation of systole is greater than can be accounted for by the lengthened cycle. As arterial pressures increase in successive segments the primary peak, *c*, due to inherent vibrations of the arterial system diminishes and



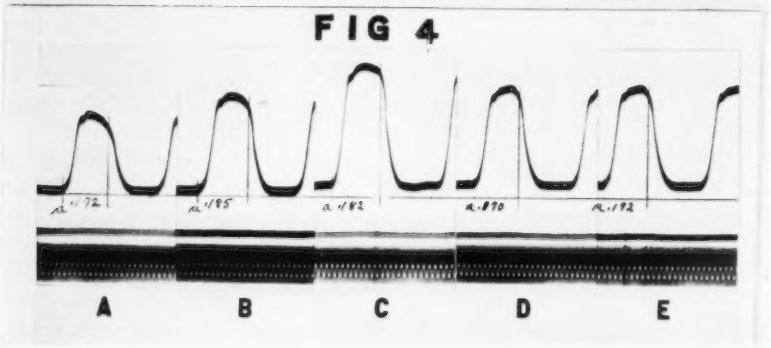


Fig. 3. Pressure pulses from subclavian artery showing successive changes before, during and after infusion of sedimented corpuscles. Letters on segments A, B, C, etc., correspond to times indicated by same letters in figure 2. Time 0.02 second. Discussion in text (expt. AA71).

Fig. 4. Pressure curves from left ventricle before, during and after infusion of sedimented corpurcles showing increase in initial tension at *a* and prolongation of systole. Time 0.02. Discussion in text (expt. AA62).

the systolic summit, *c-d*, gradually alters from a declining to a mounting summit. Such changes could either be due to a more prolonged delivery of blood during the ejection phase or to the increasing resistance offered by the more viscid blood. Since, however, the slope of the diastolic decline of pressure shows no essential deviations in segments A-D, it may reasonably be concluded that a more prolonged ejection of larger quantities of blood is the chief determining factor. The absence of changes in diastolic slope is contrary to anticipation and can only be explained by a compensatory dilatation of peripheral arterioles or by opening of new blood channels. This is consonant with the observations of Meek and Eyster (1922) and also those of Ray and Thomas (1933) who found that the surface temperature increased appreciably in similar experiments. During the beats which follow the completion of infusion (E, F) the cycle lengthens still more. The pulse pressure diminishes though it still exceeds that of the control record (A). The diastolic gradients in segments D, E and F (fig. 3) are now more horizontal, a phenomenon that can only be interpreted as showing that further reduction of peripheral resistance does not occur. This may be due to the fact that the initial dilatation of vessels and opening of new circuits has possibly reached a limit. However, the viscosity of the circulating blood doubtless also continues to increase, both because contraction of the spleen tends to return more concentrated blood to the circulation and because the continuing diuresis continues to reduce the plasma volume. The occurrence of both phenomena is well illustrated by the graphs of figure 2 (D, E, F).

During the additional hour that observations were continued, a more stabilized state obtains. The chart of figure 2 shows that the spleen volume gradually returns to normal. Effective venous pressures slowly decrease, though they still exceed the normal. The corresponding pressure pulses shown in segment F, G, H, reveal a progressive reduction in pulse pressure, though systolic and diastolic pressures continue above normal levels. The diastolic gradient remains more horizontal, indicating that even after this long period no new mechanisms are introduced for the reduction of peripheral resistance. This combination of phenomena, namely, high diastolic pressure, larger pulse pressure and reduced diastolic gradient can only be interpreted to mean that the systolic discharge of the ventricle remains augmented. The persistence of a lengthened isometric phase, together with an elevation of effective venous pressure is evidence for the belief that this increased discharge is chiefly produced by increased diastolic filling.

Effects on intraventricular pressure. In order to check the foregoing interpretation of the cardiac response a number of experiments were performed in which left ventricular and aortic pressures were simultaneously recorded. In some of these, the vagi nerves were also cut, thus

avoiding heart rate changes that complicate experiments on normal dogs. Typical effects are shown in the segments of figure 4. Segment A is a normal control. Segment B shows a curve recorded after infusion of 35 cc. of concentrated blood within 5 minutes. It shows a slight increase in cycle length and a definite rise in initial tension. The intraventricular pressure curve rises and falls more abruptly, reaches a higher maximum and has a more sustained summit. Duration of isometric contraction is unaltered, but the ejection period is prolonged. These deviations from the normal become progressively greater and reach a maximum at the end of infusion in 40 minutes, at which time the records reproduced in segment C were obtained. Obviously, the elevation of initial tension is the striking factor which modifies the output of the heart.

After cessation of the infusion, the pressures slowly fall again, but as shown in segment D initial and maximal intraventricular pressures are still considerably above normal 80 minutes later. The gradual decline of maximal pressure is further illustrated in segment E, a record taken 115 minutes after stopping infusion. The initial tension still remains elevated.

Discussion. The essential differences in the conclusions to be drawn from the results of Meek and Eyster and those just presented may briefly be summarized as follows: Their observations necessarily lead to the inference that the capacity of different blood reservoirs of the body can increase sufficiently to accommodate so large an increase in blood volume that venous return is not at all augmented or is only momentarily increased and hence that the cardiac discharge is not increased. Our results indicate that while an increase in the capacity of the blood reservoirs is indeed important, they are not sufficient to prevent an increased return and increased output of the heart.

Undoubtedly these differences are due almost entirely to methods of experimentation. No essential differences apparently obtained as regards total quantities infused or the rate of infusion. However, one difference consisted in our use of concentrated blood corpuscles rather than diluted, defibrinated blood or acacia saline solutions. In other words, while we produced a state of polycythemic hypervolemia in the sense of Rowntree, previous investigators were probably dealing with states of simple hypervolemia or oligocythemic hypervolemia. It can, therefore, not be assumed that the conditions produced by them apply to patients with polycythemic hypervolemia. A second difference arising out of different objectives in the two researches was that we administered the whole intended blood volume by continued slow injection, whereas Eyster and Meek allowed occasional short intervals during which x-ray photographs were made. Indeed, they purposely waited until venous pressures had returned to normal after each injection. The fact that such recovery

occurred promptly is itself evidence that the use of more viscid blood with higher corpuscular content causes different effects, for in several experiments in which an interruption of infusion was necessitated on account of experimental troubles, the venous pressures remained above normal for long periods. Unless additional adaptive mechanisms are gradually acquired in polycythemic patients *pari passu* with the increase in blood volume and erythrocyte counts increased cardiac output and some elevation of arterial pressures would seem to be inevitable. The tendency for clinical investigators to conclude that increased cardiac output is not a factor responsible for elevation of arterial pressures occasionally encountered in polycythemic patients must therefore be accepted advisedly.

SUMMARY

The plasma and erythrocyte volumes of anesthetized dogs were increased experimentally by slow infusion of sedimented corpuscles, during which pressure changes in the veins, ventricles and central arteries were followed, the latter two by use of optical manometers with a high figure of merit. In addition, alterations in urinary secretion, spleen volume, intrathoracic pressure, etc., were incidentally recorded.

The experiments demonstrated the following points: 1. While a considerable volume of blood can be accommodated by the dilatation of capillaries and venules in various blood reservoirs of the body, including the spleen, the maximum capacity available under normal conditions is not adequate to prevent an increased return to the heart during polycythemic hypervolemia. 2. The heart enclosed by its pericardium within the closed chest does not differ in its response from that of an exposed heart up to the limits imposed by very large increases in blood volumes. Evidence that the systolic discharge of the left ventricle increases is given by the larger pulse pressure, the inordinate increase in the phases of isometric contraction and systolic ejection as well as by the contour changes exhibited by the pressure pulses. These are discussed. 3. Intraventricular pressure curves obtained from exposed hearts enclosed naturally within the pericardium indicate that initial tension increases considerably, thus supplying the force by which dilatation up to the capacity of the pericardial sac can be produced. The increased initial tension and length therefore represent the coefficients which determine the steeper gradient of the ventricular pressure pulses and the prolongation of systole. 4. During the early stages of hypervolemia, the increased systolic output is mainly responsible for the elevation of arterial pressure, for the gradients of the diastolic portion of the pressure curves do not change. This indicates that the increase in peripheral resistance occasioned by greater blood viscosity is nicely counterbalanced by dilatation of peripheral arterioles and widening of the capillary beds. Our experiments supply no informa-

tion as to whether this involves active changes in the caliber of peripheral vessels or is entirely passive. When the polycythemic hypervolemia becomes extreme, however, the effects of increased viscosity dominate and an increased peripheral resistance undoubtedly occurs. This is shown by the changing contour of the systolic portion of the pressure pulses and by a slower gradient of the diastolic decline as well.

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ACTION OF ACETYLCHOLINE ON THE INTESTINE

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The action of acetylcholine on the isolated intestine has not been extensively studied. Takeda (1) showed that epinephrin and atropin antagonized acetylcholine on the isolated rabbit intestine. Arieff (2) studied its action on the isolated cat intestine, and Gasser (3) on the plexus free cat intestine. On other smooth muscle organs, Rydin (4) showed the antagonism of acetylcholine and nicotine on the rabbit uterus and Villaret, Justin-Besançon and Vexenat (5) the antagonism of acetylcholine and atropin on the isolated bronchial muscle of the pig.

It was therefore of interest to study the action of acetylcholine on the intestine more closely especially in its relation with other drugs and to compare its action with that of histamin. The reaction of the intestine to epinephrin, atropin, and nicotine after contraction by acetylcholine is different from its reaction after contraction by histamin. The results showed that after histamin, the action of nicotine or epinephrin was more marked than after acetylcholine. The action of atropin, however, was greater after acetylcholine than after histamin.

EXPERIMENTAL. The ileum of the guinea pig was used. The animal was starved twenty-four hours previously, lightly anesthetized with ether and decapitated. A strip about 2 cm. long was tied off at both ends with the lumen empty and suspended by means of platinum hooks in 45 cc. of salt solution in a Burn and Dale apparatus. The salt solution contained 9 grams NaCl, 0.2 gram KCl, 0.2 gram $CaCl_2$, and 0.1 gram $MgCl_2$ per litre, and was aerated with carbon dioxide free air and kept at a temperature of 37°C. The drugs were made up in the following concentrations: Acetylcholine iodide (Hoffman-LaRoche) 0.5 mgm. in 10 cc.; atropin sulphate (Eli Lilly), 0.3 mgm. in 30 cc.; histamin acid phosphate (British Drug Houses) 2.0 mgm. in 10 cc.; nicotine (Eastman Kodak Co.) 400 mgm. in 10 cc.; and epinephrin (Parke-Davis) in 1/1000 solution containing a little chloretone which did not interfere with the action. The nicotine was neutralized with HCl to bring it to the pH of the salt solution; namely, 7.8.

The interaction of acetylcholine and epinephrin. Figures 1 and 1a show the action of epinephrin on an intestinal strip contracted by acetylcholine and by histamin. The first thing to be noted is that the concentration of

acetylcholine does not affect the relaxation by a given amount of epinephrin. This is seen in the first two curves of figure 1. This fact is to be expected as the two drugs are acting on different systems or receptors in the intestine and cannot displace one another. As has been shown (6) the relaxation by

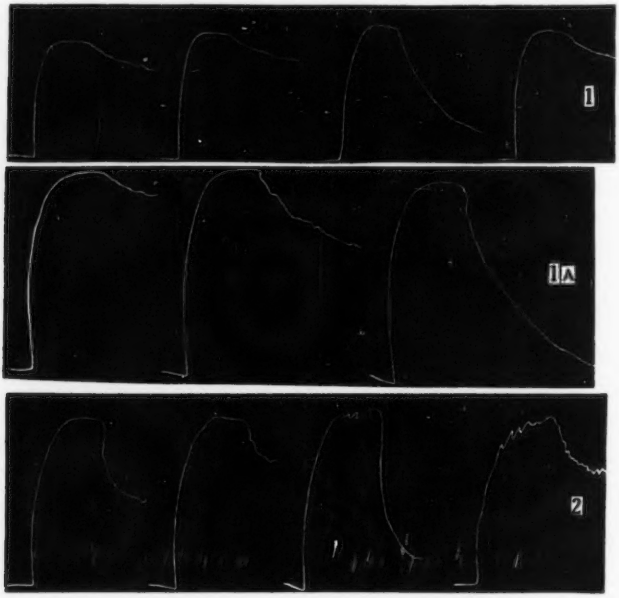

Fig. 1. The first curve represents the contraction of the ileum with 0.1 cc. acetylcholine and relaxation with 0.2 cc. epinephrin. The second curve, 0.2 cc. acetylcholine and 0.2 cc. epinephrin. The third curve, 0.2 cc. histamin and 0.2 cc. epinephrin. The fourth curve, 0.2 acetylcholine and 0.2 epinephrin. One-half minute by stopwatch between the addition of the drugs. The intestine was washed once after each contraction.

Fig. 1a. Each curve represents the contraction of the ileum by 0.2 cc. acetylcholine. The first curve shows the relaxation with 0.2 cc. epinephrin; the second, with 0.5 cc. and the third, with 1.0 cc. Conditions the same as in figure 1.

Fig. 2. The first curve represents the contraction of the ileum by 0.1 cc. acetylcholine and relaxation with 0.5 cc. nicotine. The second curve, 0.2 acetylcholine and 0.5 nicotine. The third curve, 0.2 histamin and 0.5 nicotine. The fourth curve, 0.2 acetylcholine and 0.5 nicotine. Conditions the same as in figure 1.

epinephrin after histamin is independent of the concentration of histamin for the same reason. The third curve of figure 1 shows the relaxation by the same amount of epinephrin after contraction by histamin. The relaxation is much greater. The relative insensitivity of the intestine to epi-

nephrin after acetylcholine is in agreement with the fact that epinephrin causes a further contraction and very little relaxation after parasympathetic drugs such as pilocarpine and physostigmine (7). Figure 1a shows that relaxation by epinephrin after acetylcholine is a function of the concentration of epinephrin and that complete relaxation is possible, but only with a great disparity in the concentrations of the drugs. The final concentrations for complete relaxation were: acetylcholine, 1 part in 1,800,000 and epinephrin, 1 part in 45,000.

The interaction of acetylcholine and nicotine. The first two curves of figure 2 show the relaxation of the intestine by nicotine after contraction by different amounts of acetylcholine. It will be seen that the relaxation after 0.2 cc. of acetylcholine is only half of that after 0.1 cc. although the same amount of nicotine was used in both cases. This result is also to be expected as nicotine acts on the parasympathetic system and is thus competing with the acetylcholine for the available receptors. Consequently, increasing the amount of acetylcholine within limits will decrease the effectiveness of the nicotine and the converse is true if the nicotine concentration is increased. The third curve in figure 2 shows that the action of nicotine is much more effective after histamin than after acetylcholine even though the histamin concentration is about five times greater than the acetylcholine. It has been shown (8) that there is a quantitative antagonism between histamin and nicotine thus proving that nicotine must act on smooth muscle directly. The results with acetylcholine show that although nicotine acts on the parasympathetic system, it acts much more effectively on smooth muscle. The amount of nicotine to produce any relaxation is, however, very large in comparison with the amounts of histamin or acetylcholine. The final concentrations were: histamin, 1 part in 400,000; acetylcholine, 1 part in 1,800,000; and nicotine, 1 part in 550.

The interaction of acetylcholine and atropin. The intestine contracted with acetylcholine is much more sensitive to atropin than when it is contacted with histamin. It has been shown (6) that there is a quantitative antagonism between histamin and atropin and that atropin must therefore act directly on smooth muscle. The results with acetylcholine show that its acts much more powerfully on the parasympathetic system. Thus a final concentration of atropin of 1 part in 6,750,000 causes complete relaxation of the intestine contracted with acetylcholine in concentration of 1 part in 900,000. The action of atropin is peculiar in that it either acts to cause complete relaxation or is completely inactive. Thus in concentration of 1 part in 13,000,000 it has little or no action. This is in marked contrast with the action of atropin after histamin. A concentration greater than that of histamin is required before any relaxation occurs and using 1 part in 400,000 histamin a concentration of atropin of 1 part in 50,000 is necessary to produce complete relaxation.

The action of calcium. It has been shown (6) that when the intestine is contracted by histamin the contraction is maintained indefinitely, but in the absence of calcium although the contraction reaches the same height, immediate relaxation sets in. In the case of acetylcholine contraction is also maintained indefinitely, but this is also dependent on the presence of calcium. But contrary to the histamin the height of contraction is also somewhat diminished. This is probably due to the fact that the intestine reacts more slowly to acetylcholine than to histamin and that relaxation begins before the contraction is completed, the net result of which would be a lowering in the height of contraction. Evidently calcium is necessary for the maintenance of contraction whether the drug acts on the parasympathetic endings or the muscle.

DISCUSSION. Histamin and acetylcholine show interesting parallelisms in their relation to other intestinal drugs. Epinephrin relaxes the intestine after it has been contracted by either drug and the relaxation is independent of the amount of histamin or acetylcholine and only dependent on the amount of epinephrin added. The reason for this is that epinephrin acting on the sympathetic endings does not compete with either acetylcholine acting on the parasympathetic system or histamin acting on the muscle. But the intestine is much more reactive to epinephrin when it has been contracted by histamin than by acetylcholine. The reason for this is not obvious, but it is interesting in view of the findings of Dragstedt and Huffman (9) who showed how comparatively insensitive the normal intestine is to epinephrin. If acetylcholine is always acting on the intestine, the above results would explain this insensitivity.

The relation between acetylcholine and nicotine is comparable to the relation between histamin and nicotine. In both cases nicotine is a quantitative antagonist; i.e., the extent of the relaxation is not only the function of the concentration of nicotine, but also a function of the concentration of histamin or acetylcholine. This means that nicotine is acting both on smooth muscle and the parasympathetic endings, but if the amount of relaxation is a criterion, its action on smooth muscle is much more effective than on the parasympathetic endings.

In its relation to atropine acetylcholine differs from other parasympathetic drugs in the following respect. It is very difficult to cause the isolated intestine to react to either pilocarpine or physostigmine once atropin has been added, no matter how well the intestine is washed. But it will react immediately to acetylcholine with just one washing after atropin. Despite this, it is a much more powerful antagonist to acetylcholine than to histamin.

SUMMARY

1. There is a quantitative antagonism between acetylcholine and nicotine on the guinea-pig intestine as there is between histamin and nicotine, indi-

cating that nicotine acts on the parasympathetic endings as well as on smooth muscle. The intestine is much more sensitive to nicotine after histamin than after acetylcholine.

2. The intestine is also much more reactive to epinephrin after histamin than after acetylcholine. The relaxation by epinephrin after acetylcholine is function of the concentration of epinephrin and independent of the concentration of acetylcholine.

3. Atropin in dilutions of about 1 part in 7,000,000 causes complete relaxation of the intestine contracted by acetylcholine.

4. Calcium is necessary for the maintenace of the contraction caused by acetylcholine.

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OXIDATION OF ACETYLCHOLINE BY TISSUES

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Acetylcholine is rapidly destroyed in the animal body. This has been attributed by Engelhart and Loewi (1) to the action of the esterase of the blood splitting off the acetyl group and leaving choline which is physiologically much less active. This destruction of acetylcholine by the blood has also been found by Plattner and Bauer (2). Acetylcholine, according to Ahlgren (3), augments tissue respiration and in extending this observation we have found that acetylcholine is definitely oxidized by various tissues and that a definite amount of oxygen is taken up indicating a specific oxidation. The oxidation only takes place after the splitting off of the acetic acid, but it is the choline and not the acetic acid that is oxidized.

EXPERIMENTAL. The Barcroft-Warburg apparatus was used. Rat's liver was chopped with scissors and ground in a mortar with approximately 1 cc. phosphate buffer pH 7.2 for each gram of liver and then pressed through muslin; 0.5 cc. of the resulting suspension was used in each vessel. Acetylcholine iodide (Hoffmann-LaRoche) was weighed out and dissolved in buffer so that 0.1 cc. contained 1 mgm. On adding it to the liver at 37°C. a rapid oxygen uptake occurred which stopped sharply when 63 to 66 cmm. of oxygen were taken up per milligram of acetylcholine iodide. Figure 1 shows the oxygen uptake for 1 mgm. and 2 mgm. of the substance. It will be seen that the uptake, which is calculated by subtracting the uptake of the liver alone from that of the liver plus acetylcholine, is exactly proportional to the amount of substance added. That is, the acetylcholine is not stimulating the oxygen uptake of the liver but is being oxidized. Assuming that one molecule of acetylcholine would be oxidized by one atom of oxygen the theoretical uptake for 1 mgm. of acetylcholine is 42 cmm. The actual uptake was 64 cmm. or almost exactly $1\frac{1}{2}$ atoms of oxygen per molecule of substance. Obviously the oxidation is not a straightforward one and must involve some sort of condensation.

It was necessary to determine if the iodide ion or the acetic acid liberated by the liver esterase had any effect on the oxygen uptake. In order to do this equivalent amounts of KI and sodium acetate were added to liver suspension, but neither was found to have any effect on the oxygen consumption. The choline therefore must be oxidized.

Sodium fluoride is a well known poison to esterases and recently Stedman and Stedman (4) have shown that physostigmine inhibits liver esterases. Either of these two substances added to liver, the fluoride in a 2 per cent concentration or 1 mgm. of physostigmine for every 0.5 cc. of liver, completely inhibited the oxidation of the acetylcholine. It was necessary to add the physostigmine to the liver some ten minutes before the acetylcholine in order to obtain complete inhibition. These experiments prove that hydrolysis of the ester linkage is necessary before oxidation occurs.

A pharmacological assay was made on the guinea pig intestine after the oxidation was complete. The liver plus the oxidized product was im-

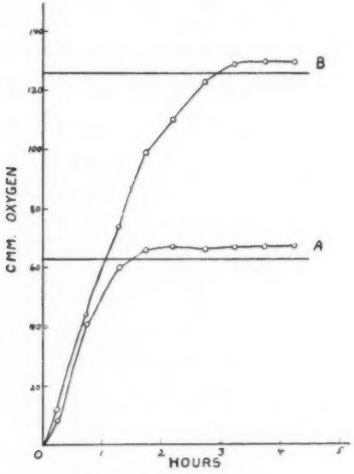

Fig. 1. Curve *A* represents the oxygen uptake of liver alone subtracted from the uptake of liver plus 1 mgm. acetylcholine iodide.

Curve *B*, the uptake of liver alone subtracted from the uptake of liver plus 2 mgm. acetylcholine iodide. The horizontal lines represent the theoretical uptake if 1½ atom of oxygen is used per molecule of acetylcholine.

mersed in boiling water for two minutes and the coagulated protein filtered off. The filtrate when added to the intestine caused no contraction, whereas the filtrate from a control to which an equal amount of acetylcholine was added immediately before boiling caused a marked contraction.

The pH optimum for the oxidation was approximately determined by using buffers at pH 6.0 and pH 7.8. At pH 6.0 the reaction was inhibited completely and at pH 7.8 the rate about one-half of that at pH 7.2. This gives an approximate pH optimum of 7.0 to 7.2. The effect of KCN was also tried. Although the oxygen uptake of the liver was almost completely inhibited by N/200 KCN, the oxidation of the acetylcholine went on at the

same rate. This oxidation then along with the oxidation of proline, xanthine and tyramin is not dependent on iron in any form.

It was interesting to see whether methylene blue could be substituted for oxygen as a hydrogen acceptor. Modified Thunberg tubes were used and liver and acetylcholine in the same proportions were added plus 1 cc. of 1/5000 methylene blue. The tubes were evacuated and allowed to stand at room temperature. The reduction time with and without acetylcholine was exactly the same indicating that this system is unable to use methylene blue. This fact places the catalyst responsible for the oxidation in the class of aerobic oxidases.

Other tissues were also tried. Rat kidney, cat liver and kidney will also oxidize acetylcholine, the last two more slowly. Guinea-pig liver, however, is entirely inactive. The enzyme causing the oxidation is fairly stable and the liver preparation will remain active for at least twenty-four hours in the ice chest.

Discussion. It is interesting that of the two drugs found in the animal body, namely, histamin and acetylcholine, both are capable of being destroyed in the body by oxidation. No definite measurement of the oxygen uptake by the histamin-histaminase system (Best and McHenry, 5) has however been made. Tyramin may also be considered in this group, for it probably is being continuously absorbed from the intestine, and it has been shown by Hare (6) and Bernheim (7) that it is definitely oxidized by liver. The oxidation of acetylcholine resembles that of tyramin in that neither are inhibited by KCN and neither are able to use methylene blue as a hydrogen acceptor. The oxidation of histamin is, however, inhibited by cyanide (Best and McHenry).

SUMMARY

1. Acetylcholine is oxidized by rat liver. One and a half atom of oxygen is taken up for every molecule of acetylcholine.

2. Physostigmine and sodium fluoride inhibit the oxidation indicating that hydrolysis of the ester linkage is necessary before oxidation will occur.

3. Methylene blue is not reduced and KCN in a concentration of N/200 does not inhibit.

4. The pH optimum is about 7.

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SIMULTANEOUS STUDY OF THE CONSTITUENTS OF THE SWEAT, URINE AND BLOOD; ALSO GASTRIC ACIDITY AND OTHER MANIFESTATIONS RESULTING FROM SWEATING

X. Basic Metals

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The fact that little attention has been given to the ascertaining of the presence of the basic metals in the sweat has prompted us to make a thorough investigation of these constituents. It has been a long established fact that sodium is present in the form of chlorides, and from our extensive study of the chlorides (1) we have estimated by indirect methods that this metal is found usually from 350 to 400 mgm. per 100 cc.

The possibility of the presence of other common metals of the body in sweat has attracted scarcely any attention. We reported for the first time the presence of calcium at the meeting of the American Physiological Society at Montreal in 1931. In a very recent article, Fishberg and Bierman (2) report both the presence of calcium and potassium from pathological cases. We fail to find any report of the presence of magnesium previous to the one we made at the meeting of the Physiological Society at Philadelphia in 1932.

We have felt that in all of our analyses of the sweat it was highly important that the skin should be thoroughly cleansed and that there should be no chance that any contamination could possibly come from the containers for the sweat. To that purpose our rubber jackets were tested for such possible sources of error. The method of collecting the sweat was the same as heretofore reported by Talbert (3).

During the summer of 1932 Haugen of this Laboratory, using the method of Jacobs and Hoffman (4), made 102 determinations on five different subjects. He found potassium constantly present in quantities ranging from 28.3 to 145.4 mgm. per 100 cc. of sweat. The latter figure is rather high; in reality an average of 45 to 50 mgm. was more common. Fishberg and Bierman (2), while using a different system for calculations, have obtained a higher concentration. Whether this difference might be explained from the fact that they were using pathological subjects or from the difference in the method of collecting the sweat is a matter of some conjecture.

The determination of calcium was first made in this Laboratory by Bryant during the summer of 1930 using the colorimetric method of Roe and Kahn (5) and was further extended by Carpenter in 1931, using not only the same method, but frequently checking by the gravimetric method of McCrudden (6) with agreements that were fairly satisfactory. There were in all 210 determinations yielding as low as 0.36 mgm. to a maximum of 11.8 mgm. per 100 cc. More frequently the concentration was near to 5 mgm. per 100 cc. In three determinations there were negative results.

As for magnesium, Carpenter, using the method of McCrudden (6), found this metal in 79 determinations ranging from 0.14 to 4.5 mgm. per 100 cc. He used five different subjects and in only two instances did he find negative results.

CONCLUSIONS

The sweat secretes about ten times as much sodium as potassium and about five times as much calcium as magnesium. However, there was at times a surprising variation in the relative amounts of the last two named; indeed, in rare instances magnesium was secreted in greater concentration than the calcium.

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THE EFFECT OF OCCLUSION OF THE CAROTID ARTERIES ON HEART RATE AND RESPIRATORY RATE BEFORE AND AFTER DENERVATION OF THE CAROTID SINUS IN NORMAL DOGS

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"I tied the carotid arteries. Respiration was somewhat quickened and the heart's action increased, but no other effect produced." This fundamental observation was made by Sir Astley Cooper as early as 1836. Since that time various explanations have been offered for these changes in the respiratory and pulse rates.[1] François Frank (1880) concluded that the increased pulse rate was due to a direct action on the accelerator center while Hunt (1899) explained the results by a decrease in vagal tone. Kisch and Sakai (1923) proposed both an increase in accelerator tone and a decrease in vagal tone. Hering (1924) maintained that the primary effect was peripheral, the decrease in pressure stimulating the end organs in the carotid sinus which reflexly caused an increase in the heart rate. The increase in the respiratory rate has also been explained on the basis of a central mechanism (Schmidt, 1928) and a peripheral one (Heymans and Bouckaert, 1930). However, Schmidt clamped both the carotid and vertebral arteries.

All of this work has been done on animals which were either anesthetized or subjected to severe operative procedures or both. The profound effect of anesthesia and acute operations on respiratory reflexes may be well illustrated by the experiments of Wright (1930). By clamping both the vertebrals and carotids in each case he obtained in cats under chloralose anesthesia a decrease in depth and rate of respiration and exactly the opposite effect in a decerebrate animal.

Accordingly, we decided to make an analysis of the effect of clamping the common carotid arteries of normal unanesthetized animals which had been trained to lie quietly throughout the experiment. For comparative purposes, a few additional studies were made on dogs under ether or morphine anesthesia.

[1] As the literature for this subject has been reviewed in detail by Hering (1927) and Heymans (1929) a complete historical summary is not necessary for this paper.

METHODS. In order to make the carotids available for clamping, the following operation was performed. Both carotid arteries were freed from their sheaths for a length of about 7 cm., care being taken not to approach the region of the bifurcation of the carotids during this dissection. These arterial loops were held in position by skin flaps sutured together underneath the arteries. To facilitate this suturing and to avoid as much as possible handling the carotids, a large hemostat supplied with two T-shaped tips was used. The latter were found to be most convenient when constructed with the tops of the T's 4 cm. long. By grasping the skin flaps from the outside with this instrument, the arteries could be held in place with ease and the suturing was rapid and safe. After healing of the incision, either or both of the carotids could be occluded by compression of the loops and their contained arteries between the fingers.

Records of heart rate were taken with an electrocardiograph. One large zinc electrode was placed over the chest and another on a hind leg. Respiratory rates were also included on the electrocardiographic film by means of a pneumatic pneumograph. Any muscular movements which would have affected the heart or respiratory rates could be instantly noted by aberrant movements of the shadows in which case the experiment was discontinued.

When the heart rate had reached a constant level, a two minute record was taken. For the first thirty seconds normal heart and respiratory rate was registered. Without interrupting the record, one or both of the carotids were clamped, and kept occluded for sixty seconds. Removal of the carotid clamp was followed by another thirty second normal record. This gave an uninterrupted record of the pulse and respiratory rate before, during and after clamping. Similar experiments were also made under morphine or ether anesthesia.

After a series of such preliminary experiments, the animals were subjected to a second operation. The bifurcations of both carotid arteries were exposed, care being taken not to disturb the arterial loops lower in the neck. All tissue adherent to the arteries was dissected away, the branches being practically stripped of their adventitial layers for a distance of almost 1 cm. from the bifurcation. The cleaned arteries were painted with 88 per cent phenol which was then neutralized well with 70 per cent alcohol and the whole region was washed with normal saline.

After recovery from this operation, a series of clamping experiments was performed similar to those before denervation. In order to see if clamping the skin had any effect on heart and respiratory rates, records were taken during which the arteries were not clamped, the skin in the region of the loops being pinched with the same force as used in clamping. This procedure had a negligible effect on pulse and respiratory rate as is shown under control experiments in figures 1 to 4.

Results. The results of experiments on four dogs are given in graphic form in figures 1 to 4 and a detailed report of the effects on one dog in table 1. In the table and the figures, the results appearing in the column "average change" were obtained by averaging the change produced by clamping with that produced by unclamping for each experiment. Thus the increase of rate on clamping and decrease on unclamping are considered as plus values, while decrease on clamping and increase on unclamping are considered as minus values. Consequently, the figures in the column "average change" indicate both the qualitative and quantitative effect of clamping in a given experiment.

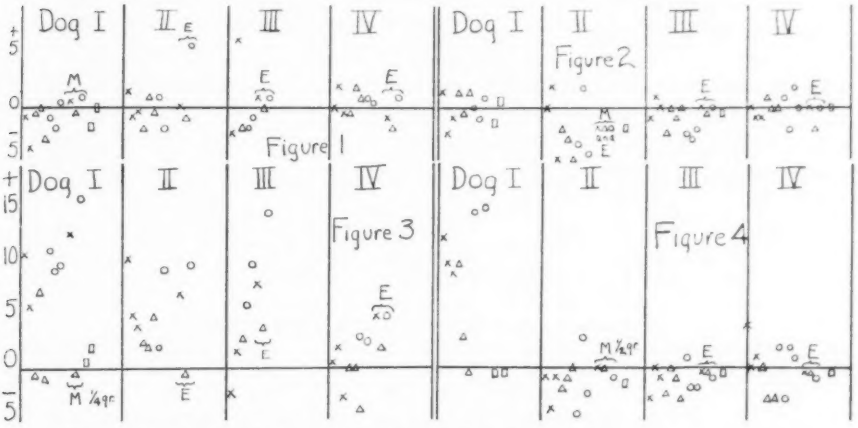

Fig. 1. Average respiratory rate change before denervation.

Symbols: ×, right carotid clamped; △, left carotid clamped; ○, both carotids clamped; □, control experiment; *E*, dog under ether anesthesia; *M*, dog under morphine anesthesia.

Fig. 2. Average respiratory rate change after denervation.

Fig. 3. Average pulse rate change before denervation.

Fig. 4. Average pulse rate change after denervation.

Respiratory rate. Clamping the common carotid arteries in our intact unanesthetized animals produced no definite change in respiratory rate (fig. 1). The maximum change which occurred in the experiments was 6½ respirations per minute and a change of this order was found in only one of the twenty-nine experiments performed. Indeed, if an average be taken of the figures expressing the effect of clamping in the twenty-nine experiments, the result is −0.4 respiration. Consequently, although small changes of rate were found in two out of the series, no consistent effect of any magnitude indicative of reflex action was obtained. The experiments performed under ether or morphine anesthesia yielded similar results.

Denervation of the carotid sinuses did not alter the result on respiratory rate produced by clamping the carotid arteries (fig. 2). Three experiments in the series of thirty-eight showed changes of as much as −4 respirations per minute, while the average was −1 respiration per minute. Again ether anesthesia had no influence on the effect produced by clamping, the average of nine experiments being −1 respiration per minute.

TABLE 1

Dog III

DATE	CAROTID CLAMPED	CONDITIONS	HEART RATE PER MINUTE				RESPIRATORY RATE PER MINUTE			
			Before	Clamp	After	Average change	Before	Clamp	After	Average change
1931										
Dec. 11	R	Normal	62	60	63	−2½	14	12	15	−2½
12	R	Normal	62	62	59	+1½	20	28	23	+6½
11	L	Normal	64	67	64	+3	14	13	16	−2
11	Both	Normal	62	70	66	+6	14	12	14	−2
12	Both	Normal	58	66	54	+10	22	22	24	−1
1932										
Jan. 21	R	Ether	216	224	—	+8	44	44	42	+1
21	L		210	216	214	+4	44	48	52	0
21	Both	Anesthesia	184	201	188	+15	44	47	48	+1
Denervation of both carotids, April 18, 1932										
April 25	R	Normal	86	83	86	−3	14	13	14	−1
May 4	R	Normal	88	85	82	0	22	23	22	+1
9	R	Normal	92	89	88	−1	20	19	18	0
April 25	L	Normal	81	79	82	−2½	15	12	14	−2½
May 4	L	Normal	86	84	82	0	24	23	22	0
4	L	Normal	86	81	78	−1	26	24	24	−1
9	L	Normal	86	83	86	−3	16	17	18	0
April 25	Both	Normal	82	81	78	+1	14	12	15	−2½
May 4	Both	Normal	80	80	84	−2	26	24	28	−3
9	Both	Normal	84	83	86	−1	18	16	18	−2
13	R	Ether	180	181	183	−½	52	56	60	0
13	L		192	196	201	−½	62	57	51	−½
13	Both	Anesthesia	206	210	216	−1	56	51	46	0
9	—	Control	78	79	81	−½	15	16	18	−½

Heymans and Bouckaert (1930) reported increases of as much as 16 respirations per minute by clamping both common carotid arteries in anesthetized, vagotomized dogs. Schmidt (1932) found that the respiratory stimulation following occlusion of the carotids was usual but not constant in acute experiments. Since the rise was not always abolished by sinus denervation he concluded that other factors besides the sinus reflex were

responsible for this effect. In our experiments it is definitely shown that changes in pressure in the carotid sinus produced by clamping the carotid arteries in normal dogs have no marked effect on the respiratory rate.

Pulse rate. In contrast to the lack of change of the respiratory rate, occlusion of the common carotid arteries, either singly or both together resulted in a definite increase in heart rate (fig. 3). Usually, the effect obtained by clamping both arteries was greater than that obtained by clamping a single vessel. Heymans (1929) recorded isolated experiments in which increases of about twenty-five beats per minute were obtained by clamping both common carotid arteries in anesthetized vagotomized dogs. However, the greatest increase in our intact unanesthetized animals was $10\frac{1}{2}$ beats per minute (2 experiments). The average of the twenty-nine experiments was +4 beats per minute.

Ether anesthesia caused a slight increase in the effect produced by clamping. In nine experiments under these conditions the average heart rate was +6 beats per minute. It should be noted that the resting heart rate in the normal dogs averaged 70 beats per minute as contrasted to an aver-

TABLE 2

Average resting pulse rates

	DOG I	DOG II	DOG III	DOG IV
Before denervation	71	73	62	75
After denervation	66	77	85	89

age rate of 200 beats per minute on anesthetized animals. Thus the heart was working at an entirely different level in the two experiments. An increase of fifteen beats was obtained on clamping both carotids of an animal under morphine.

Denervation of both carotid sinuses eliminated the change in heart rate resulting from clamping the carotid arteries in three animals (fig. 4). Due probably to incomplete denervation this response was not abolished in dog I, the first animal on which the operation was attempted. Ether anesthesia did not alter the results of clamping the carotids in the denervated animals. This indicates that the increased changes produced by anesthesia before operation resulted from a direct action on the sinus reflex.

As previously observed by Heymans (1929) in acute experiments, it is worthy of note that the resting heart rates in two dogs were somewhat higher after denervation than before (table 2). Since the results on the other two dogs were negative no definite conclusions can be drawn from this series.

The only other experiments that have been made on normal animals in

this connection were made by Cromer and Ivy (1931) and have not been reported in detail. They studied the effect of exercise on pulse and respiratory rates and blood pressure in dogs before and after carotid sinus denervation and found no significant difference in the reaction under the two conditions. The difficulty of making an exact comparative study of the recovery periods of exercise in animals is well known. We feel that we have obtained a better analysis of the carotid sinus mechanism in normal animals by using resting dogs.

SUMMARY

Clamping the carotids in normal unanesthetized dogs produced a rise in the pulse rate. This rise was abolished in three dogs by denervation of the carotid sinus. In one dog the rise persisted after denervation. Ether anesthesia increased slightly the magnitude of the rise during clamping but this effect was also abolished by denervation in three dogs. The average resting pulse rate was higher in two dogs after denervation than before.

Clamping the carotid arteries produced practically no change in the respiratory rate either before or after denervation of the carotid sinus.

The authors wish to thank Dr. E. K. Marshall, Jr. for his many helpful suggestions during this work.

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STUDIES ON THE PHYSIOLOGY OF SLEEP

VIII. Diurnal Variation in Performance[1]

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That diurnal sleep is a habit depending upon the cerebral cortex for its development and persistence was concluded from the observations of Kleitman and Camille (1932) on the sleep of decorticated dogs, but the mechanism underlying the establishment and maintenance of this habit remained to be elucidated. It is well known that the diurnal alternation of sleep and wakefulness is one of several periodic variations in the activities of the organism that show a 24-hour cycle. These changes may be the cause of diurnal sleep or they may result from it, but they may also be merely accompanying it, the sum total of the variations, including sleep and wakefulness, being manifestations of a diurnal rhythm in the organism that adapted its activity and rest to the alternations of day and night. The fact that some of these changes, such as the diurnal variation in body temperature, persist when one goes without sleep for a night may be hastily interpreted as indicating that these changes are not connected with sleep, but it was shown by Galbraith and Simpson (1904) and by Toulouse and Piéron (1907), among others, that the diurnal temperature curve may be inverted in man and higher animals by making them stay up at night and sleep in the day-time for a few weeks. Thus the body temperature has a definite relation to rest and activity, but once the temperature rhythm has been established it cannot be easily upset. The purpose of this investigation was to determine whether or not diurnal sleep can be brought into a causal relationship with some of the other diurnal changes.

Subjectively the diurnal sleep habit manifests itself in a state of drowsiness which gradually overtakes one in the evening, leading to a disinclination to continued wakefulness. Depending upon the regularity of one's daily routine this drowsiness may or may not appear at a definite hour, may or may not be allowed to develop to the point of practical irresistibility. Usually one coöperates by retiring into a quiet dark room and lying down on a comfortable couch, sleep readily supervening. Under unusual

[1] Aided by a grant from the Rockefeller Foundation Biological Fund, University of Chicago.

conditions, when varied events in the environment cause a continued stream of impulses to reach the central nervous system from the different sense-organs, the onset of sleep may be delayed far beyond the customary hour of retiring.

The probable importance of muscular relaxation, or a decrease in the number of proprioceptive impulses to the nervous system, in producing drowsiness and sleep has been discussed repeatedly (Kleitman, 1929). If it could be established that there existed a diurnal change in the tonus of the skeletal musculature we might have a basis for the interpretation of the diurnal drowsiness which is the underlying cause of the diurnal sleep habit. In a previous communication (Kleitman, 1925) it was reported that apart from the increased urinary excretion of phosphates during sleep, there was an indication of the existence of a definite curve of phosphate excretion during the usual waking hours. On the evidence that muscular activity meant retention of phosphates in the system and therefore their decreased excretion in the urine, it was suggested that "those individuals who show a definite smooth curve of phosphorus secretion, with the lowest point about noon-time, possibly possess a gradually increasing muscular tonus (efficiency?) during the early part of the day and a gradual ebbing of the tonus in the afternoon." Efforts were then made to detect other changes which would support or refute the contention that there may be a diurnal change in muscle tonus. That the body temperature curve may be looked upon as being due to such a diurnal variation was concluded from the results of Kleitman and Doktorsky (1933) on the effects of changes in the position of the body on the rectal temperature in man. Assuming the horizontal position after standing up for an hour always resulted in a lowering of the rectal temperature, and conversely, standing up after lying down invariably caused a rise in temperature. The temperature changes were ascribed to the increased muscular contraction on standing and to muscular relaxation on lying down. It was thought that apart from affecting the excretion of phosphates and the body temperature the degree of tonus in the body musculature should have an effect upon the speed and accuracy with which various manipulations were carried out, and it was decided to employ a number of tests that could be easily repeated several times during the waking period in order to discover a possible diurnal variation in such performance.

Many attempts were made to detect such diurnal variations in a variety of tests. Lombard (1887) in his extensive investigation of the knee-jerk in man found that the greatest response was obtained in the morning, right after breakfast, and that from then on there was a falling off in the height of the knee-jerk until bed-time. Dresslar (1892), however, obtained results that challenged Lombard's interpretation of his knee-jerk findings as showing a gradual development of muscular fatigue during the daily

waking period. Dresslar determined the time required to tap a telegraph key 300 times, running six tests daily, at two-hourly intervals, from 8 a.m. to 6 p.m. Speed of performance was lowest at 8 a.m., improved gradually up to 4 p.m., but fell off a little at 6 p.m. Dresslar did not study performance after 6 p.m., but this was done by Bergstroem (1894) who measured muscular efficiency from 7 a.m. to 10 p.m. and obtained a rather smooth diurnal curve of performance, with minima early in the morning and late at night and a maximum at 2 p.m. However, other tests made by Bergstroem led him to conclude that "there is no general type of daily rhythm." In the most extensive work on the subject, containing a discussion of the literature, Marsh (1906), in a variety of tests involving muscular activity, reported the same type of diurnal variation in performance as that noticed by Dresslar and by Bergstroem, but he also obtained conflicting results which he ascribed to a number of interfering factors. Hollingworth (1914) found that performance in more strictly motor tests showed a well defined diurnal variation, while in "mental" tests there was a gradual decrease in efficiency from early in the morning to the end of the waking period. He concluded that there was no evidence of "organic rhythms or diurnal factors" in the performance of the tests.

METHODS. To avoid the disturbing effects of the presence of an observer it was decided to use only such tests as could be performed on the subject by himself. To make these tests as soon as one woke up in the morning and just before going to bed, they had to be done in the subject's home and therefore without any special apparatus. Of the six subjects used, two slept in the laboratory, and they performed additional tasks requiring recording and other apparatus. The tests were made five times daily: immediately upon getting up, one hour later, just before the noon meal, just before the evening meal, and just before going to bed. Each of the subjects performed one or more tests at a time, for twenty days or more, and the average figures for the speed and accuracy of performance at different times of the day determined. One subject was made to go through a number of tests ten times daily for 20 days, as a check-up upon the validity of the diurnal curves based on figures obtained from five-tests-a-day series. In many cases there was a continued improvement in performance with the repetition of a test, but this did not influence the relationship of the average values for the different times of the day. To compare the efficiency of performance in the several tasks done during the day, the first value for speed or accuracy was considered as unity, and the reciprocals of the ratios of the later values to the first one were used as indices of performance at other times of the day. The tasks were selected from a wide list of standard tests, all involving muscular activity, either as movement or steadiness. There were no problems to solve, and the individual tests required little or no mental activity on the part of the subject.

The six tests requiring no special apparatus were:

1. Dealing a pack of cards into four hands, face of cards downward (156 cards).

2. Sorting the same pack of cards, face upward, according to their denomination.

3. Drawing a line between the double lines of a five-pointed star seen as reflected by a mirror, without touching either of the double lines,—known as mirror drawing.

4. Copying a text of five-letter nonsense syllables, 400 letters in all.

5. Transcribing a text of 400 letters into a code that had been previously learned.

6. Multiplying an eight-digit number by another eight-digit number on paper, three multiplications to a test.

In all the above tests performance was based on the time it took to accomplish a given task (measured by a stop-watch), and in the last four tests on the number of errors made. It is clearly recognized that considerably more cerebral activity is involved in tests 4, 5, and 6 than in 1, 2, and 3.

The two tests for "steadiness" were:

7. The ability to hold a metal stylus in one of several holes of varying diameter in a metal plate without touching the edges of the hole (Whipple, 1914). Any contact between the stylus and the plate closed an electric circuit which actuated a counter. Scoring was based on the number of contacts made in three one-minute trials separated from each other by one minute of rest.

8. The ability to stand upright, with eyes closed. The inevitable swaying of the body in the course of one minute was recorded by the apparatus used by Lee and Kleitman (1923).

As a rule two or more tests were run at the same sitting at the end of which the body temperature was measured by means of a clinical thermometer held in the mouth for five minutes.

Results. There was a satisfactory agreement in the results obtained on the same test in different subjects. The curves of both speed and accuracy of performance showed a well-marked diurnal rhythm, with minima early in the morning and late at night and a maximum in the middle of the day. In most cases where five tests were made daily the average figures for the noon tests were slightly higher than for the 6 p.m. tests, the actual maximum probably lying in between, but closer to the noon hour (table 1). The composite diurnal temperature curve was somewhat higher at 6 p.m. than at noon, the highest temperature being attained later than the hour corresponding to the maximum of performance. Otherwise the temperature curve was quite parallel to that of performance. Of the remaining three daily trials the performance immediately upon getting up was usually

but not always inferior to that obtained just before going to bed, an observation made by previous workers. The second morning trial, one hour after the first, yielded results practically always higher than those obtained late in the evening. For illustrative purposes the average figures for speed and accuracy of performance for 20 days in one subject, from 7 a.m. to 11 p.m., with ten tests daily instead of five, are given in figure 1. It will be seen that in this subject the curve of performance reached its maximum rather early in the day, but the same is true of the diurnal temperature curve. This parallelism was seen also in the tests of steadiness, as shown in figure 2, and in the swaying of the body.

Discussion. The results reported indicate the existence of a definite diurnal variation in speed and accuracy of performance in all the subjects and for all the tests. If efficiency of performance depends upon the tonus

TABLE 1

The average time, in seconds, required to perform certain tests and the average body temperature, in degrees Fahrenheit, at different hours of the day

TYPE OF TEST	NUMBER OF SUBJECTS	NUMBER OF DAYS	7 A.M.	8 A.M.	12 M.	6 P.M.	11 P.M.
Dealing 156 playing cards	1	29	50.1	48.2	47.1	47.4	49.8
Sorting 156 playing cards	3	69	135	129	125	127	133
Mirror drawing of star	3	69	38.4	37.5	35.3	36.3	37.6
Code transcription, 400 letters	4	64	407	362	344	350	364
Copying text of 400 letters	3	56	291	267	256	259	266
Multiplication of 8 digit number by 8 digit number	3	69	427	409	371	373	384
Body temperature (mouth)	5	108	97.64	97.71	98.21	98.33	97.73

of the muscles, and that assumption is particularly warranted in the tests for steadiness, we have evidence of a diurnal variation in the tonicity of the skeletal musculature. The previously reported data on the variation in the excretion of phosphates and in the body temperature, and the dependence of each of these upon muscular activity, serve to strengthen this hypothesis. The presence of a diurnal rhythm in the tonicity of the musculature would prove to be an important element in the mechanism of the diurnal sleep habit. The gradual decrease in muscle tonus in the evening should lead to a progressive diminution in the number of proprioceptive impulses that are important in the maintenance of the waking state. From the course of the usual temperature curve during the night it appears that the muscles continue to relax after the onset of sleep, perhaps contributing to the increase in the depth of sleep. Awakening during the night is usually followed by the immediate resumption of sleep. As the diurnal curve of muscle tonus turns up, it acts synergistically with other

internal and external changes (distention of bladder, daylight, noises) to make the individual get up after one of his chance awakenings. The tonus of the muscles is not greater just after getting up than before going to bed.

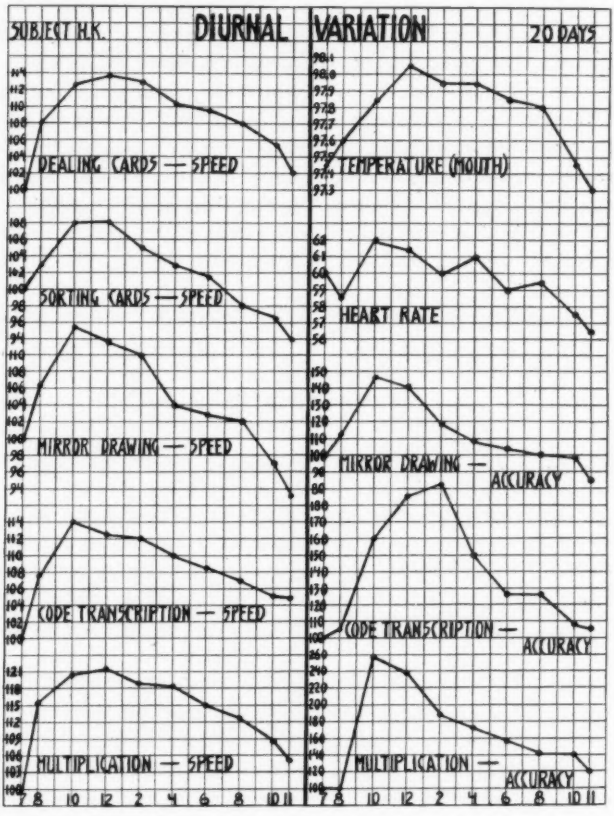

Fig. 1. Diurnal variation in speed and accuracy of performance expressed as the reciprocals of the ratios of the time it took to perform a task and the number of mistakes made to the time and the mistakes made in the first series of tests at 7 a.m., at which hour the speed and accuracy of performance are taken to be 100. One subject, 10 trials daily, the average of 20 days. The actual average figures for the 7 a.m. trials were as follows: dealing cards, 45.5 seconds; sorting cards, 96.5 seconds; mirror drawing, 17.1 seconds, 8.4 mistakes; code transcription, 291 seconds, 1.28 mistakes; multiplication, 283 seconds, 4.9 mistakes; temperature, in degrees Fahrenheit and heart rate in beats per minute are given in terms of actual figures obtained, and not as reciprocals.

It may be lower, especially in persons getting up by the alarm clock. That is why many authors report poorer performance immediately after than before a night's sleep. Marsh, undoubtedly correctly, interprets this morning inertness as a protracted drowsiness. There seems to be a "drowsiness level" in the diurnal curve of muscle tonus below which it is hard to keep awake, and the sleep period corresponds to the part of the curve that is below that level. Sleep can be produced during the customary waking period under appropriate conditions such as general inactivity and muscular relaxation, and conversely, wakefulness may be continued after the drowsiness level had been reached by activity and varied sensory stimulation.

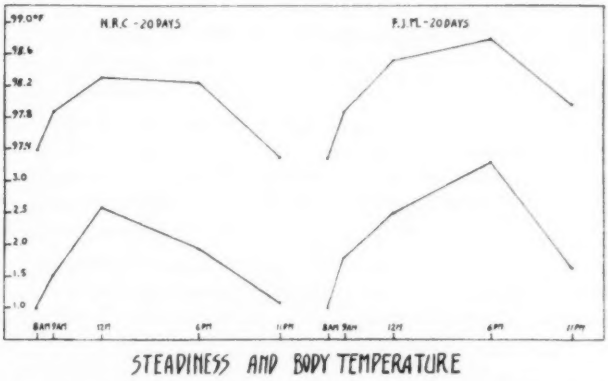

Fig. 2. Diurnal variation in steadiness expressed as reciprocals of the ratios of the numbers of contacts made in three one minute tests to the number of contacts made during the 8 a.m. trials, at which time the steadiness is taken to be 1. Two subjects, five trials daily, the average of twenty days. The actual average number of contacts for the 8 a.m. trials for N. R. C. was 27.6, for F.J.M., 21.9. Body temperature, in degrees Fahrenheit, is given in terms of actual figures obtained, and not as reciprocals. The upper curves represent body temperature, the lower, steadiness.

The diurnal variation in muscle tonus probably depends upon the animal repeatedly adapting its activity and rest to the alternations of day and night, and, as exemplified by the behavior of decorticated dogs, this adaptation does not take place in the absence of the cerebral cortex in animals naturally endowed with one. Depending upon a certain degree of complexity in cortical structure and function, the diurnal sleep habit is formed only in higher animals, and a stage in their development when cortical processes have begun to play an important part in their daily activities.

SUMMARY

1. There is a diurnal variation in the speed and accuracy of performance in man, with a maximum in the afternoon and minima early in the morning and late at night.

2. This variation, like that in body temperature and urinary excretion of phosphates, may be due to a diurnal rhythm in the tonicity of the skeletal musculature, which is probably responsible for the development and persistance of the diurnal sleep habit.

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RESPIRATORY DEATH FROM CENTRAL VAGUS STIMULATION AFTER REMOVAL OF STELLATE GANGLIA

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During the course of some experiments on the stellate ganglia and the carotid sinus reflexes, two animals died from a respiratory death under unusual circumstances. These circumstances were of such a nature as to suggest that the stellate ganglia might transmit impulses responsible for the escapement of the respiratory center from vagus inhibition or which in some way regulate the activity of the respiratory centers.

A review of the literature revealed that relatively little investigation has been directed to ascertain whether or not impulses mediated by the stellate ganglia play a rôle in the activity of the respiratory mechanism (Macklin, 1929; Ranson, 1921). Hence, a program was initiated to investigate this problem. The following experiments were performed to determine the effects of bilateral stellectomy on the escape of the respiratory mechanism from vagus inhibition.

METHODS. Dogs anesthetized with 250 mgm. of sodium barbital per kilo were used. The procedure usually followed was to dissect out both vagi from a point low in the neck up and almost to the ganglion nodosum. The vagi were then sectioned low in the neck and the stellate ganglia exposed. The central end of one vagus was stimulated continuously and the time of escapement of the respiratory center from inhibition was noted, after which stimulation was discontinued. Then one stellate was removed and the stimulus repeated until escapement occurred. Then the second stellate was removed and the stimulus repeated. This general procedure was not always followed, that is, in some instances the stellates were exposed or removed before the vagi were dissected and sectioned. In all experiments the electrode was held at one point for several seconds and then moved cephalward to avoid the possibility that escapement might be due to "burning out" of the nerve. A stimulus from a Harvard inductorium was used with the secondary coil generally either at 4 or 6 cm. and the primary coil connected with two dry cells in series. In certain instances the full coil was necessary to inhibit respiration completely and cause death.

The stellates were removed by approach through the neck which with skill may be accomplished without rupturing the pleura. In several experiments, the stellates were removed by approach through the back to ascertain if this approach involved less trauma. This method was given up because it inflicted greater trauma.

The completeness of the removal of the stellates was checked post mortem in each experiment and it was found that they had been removed with all branches down to the third, fourth or fifth thoracic rami, except in one dog. Graphic records were made of femoral arterial blood pressure, chest movements, intrathoracic pressure, and intratracheal pressure.

The experiments performed have been grouped as follows: Group I, 19 dogs, a control group in which stellates were intact and in which the vagi were stimulated centrally with the faradic current. Group II, 7 dogs, in which the stellates were removed and then the vagi stimulated within a few minutes. Group III, 8 dogs, in which the stellates were removed from one week to four months previously and then the vagi stimulated. Group IV, 9 dogs, in which the carotid sinus area was exposed prior to removal of the stellates and section of the vagi. Group V, 6 dogs, in which the vagi were stimulated up to and involving the ganglion nodosum and nerve trunks in the region of the jugular foramen.

Results. *Group I.* Although the effect on respiration of stimulation of the central end of the sectioned vagi is well known, it seemed wise to repeat some of these experiments (19 dogs) under our technique. In most cases (12 dogs) the stimulus was applied to one side only. In all nineteen cases, escapement of the respiratory center occurred in from five to one hundred seconds, the average being thirty-four seconds. The dog that showed an inhibition of one hundred seconds before escapement occurred, developed numerous extra systoles on stimulation. The second longest inhibition of respiration (60 seconds) occurred in a dog that had pneumonia. In three dogs, full coil stimulus was continuously applied to one vagus and then the other. In two, the respiratory center escaped from inhibition in 70 and 75 seconds respectively, and respiration continued in spite of the stimulus. The third died in four minutes after the second vagus was stimulated near the ganglion nodosum. The first vagus had been stimulated eight and one-half minutes with the usual respiratory escape in fifteen seconds. In a fourth dog with the secondary coil at 4 cm., the central end of one vagus was stimulated up to the ganglion nodosum and then the other. The stimulus was continued for eighteen minutes. Respiration broke through the inhibition in one minute. Abnormal breathing continued for two minutes and then ceased for one minute, and then was resumed. This change from a period of apnea to a period of abnormal respiration (Cheyne-Stokes) continued throughout the period of eighteen minutes, the blood pressure falling during the stimulation from 116 to 54 mm. Hg. The res-

piration then became normal, the stimulus was discontinued and the blood pressure returned to 116 mm. Hg.

In this connection it is significant that Mann (1918) reports that it is impossible to kill dogs by strong electrical stimulation of any of the large nerve trunks including the vagus even though the stimulus may be continued several hours. He found that death on stimulation of nerve trunks occurs only when dogs are deeply etherized. In his deeply anesthetized dogs (so deeply anesthetized that sciatic stimulation did not augment respiration), central stimulation of the vagi caused respiratory inhibition and a prompt marked fall in blood pressure which resulted in death in a few minutes. This picture differs from that which we have observed in dogs with their stellates removed as reported in the following groups of experiments. In our experiments on dogs with the stellates removed, continuous central stimulation of the sectioned vagi did not have an immediate unfavorable effect on blood pressure and heart rate except those changes incident to asphyxia. Respiration was completely inhibited except for an occasional respiratory movement or brief periods of Cheyne-Stokes respiration in some dogs.

Group II (7 dogs). Included in this group are some dogs from group I whose stellates were removed after determining the time required for the escapement of respiration from continuous central vagus stimulation. The removal of one stellate increased the period of respiratory inhibition caused by central vagus stimulation. Two of the seven dogs died when only one stellate was removed. The average time of respiratory inhibition (not including the two that died) with one stellate out was 64.4 seconds (5 dogs) as compared with 34.5 seconds (18 dogs) with both stellates intact. The remaining five dogs, on removal of the second stellate, died a respiratory death in 5.25 minutes (average) on continuous stimulation of the central end of both vagi, first one and then the other. Time was recorded from the onset of the final stimulation until the blood pressure had reached its lowest level and heart beats were no longer recorded by the manometer (fig. 1). In these dogs it was not necessary to use the entire length of the exposed vagi or to stimulate the vagi deep in the neck, or to use the full coil. This statement is made because it is possible, when the vagi are stimulated near the ganglion nodosum, that spread of current to the medulla or other nerve structures may occur and cause death. This is the reason we placed some of the experiments in group V. Intrathoracic pressure records ruled out pneumothorax as a cause of death. Pneumothorax as a cause of death was also ruled out by a post-mortem examination in which the pleura was exposed by a careful section between the ribs and the lungs could be observed in contact with the parietal pleura.

Group III. This group (8 dogs) of experiments, since the stellates were removed from seven days to four months previously, demonstrates that the

respiratory inhibition and death in the dogs of group II was not due primarily to "surgical shock" brought on by the stellectomy. It was more difficult in these dogs to obtain continuous inhibition of respiration, an occasional respiratory movement breaking through. However, a respiratory death resulted (figs. 2 and 3). The average time required to cause death in these dogs was 6.8 minutes as compared to 5.25 minutes for the dogs in group II. In two other experiments death did not result on stimulating the vagi but did occur on stimulation of the nodose ganglia. These two experiments are described later in group V.

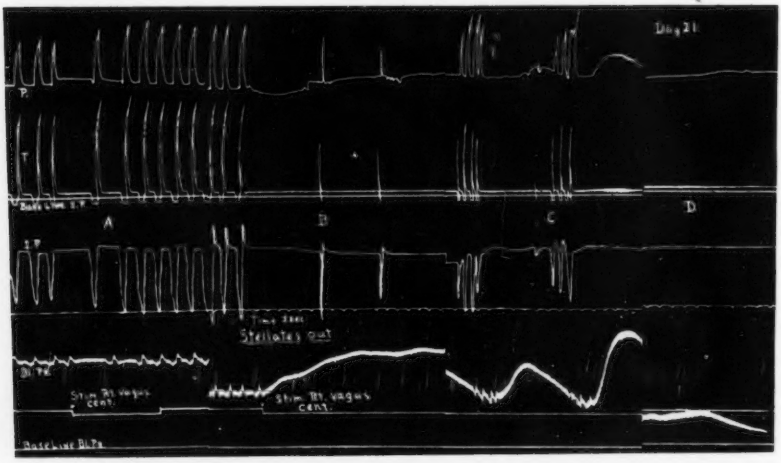

Fig. 1. Dog 21 in which the central end of the right vagus was stimulated until escapement of respiration occurred, then both stellates were removed and the stimulus repeated continuously until death. *P*, pneumograph tracing; *T*, tracheal respiration; *I.P.*, intrathoracic pressure; *Bl. Pr.*, blood pressure. Section A of tracing shows respiratory inhibition (blood pressure, 105 mm. Hg) before removal of stellates; section B, start of stimulation of central right vagus with stellates out (blood pressure 82 mm. Hg); section C, Cheyne-Stokes respiration and Traube-Hering waves in blood pressure; section D, death. This tracing is included to show that respirations may break through the inhibition produced by stimulating the vagi centrally. When a number of respirations break through as in this experiment, the period during which the vagi must be stimulated to induce death is prolonged

Group IV. This group of experiments (9 dogs) is similar to group II. They differ in that either the superior laryngeal, the glosso-pharyngeal, or the carotid sinus, or all had been dissected and stimulated previous to central vagus stimulation. Stimulation of the superior laryngeal, glossopharyngeal, or carotid sinus with the stellates out was accompanied by the normal escapement of respiration from inhibition (fig. 2). When the cen-

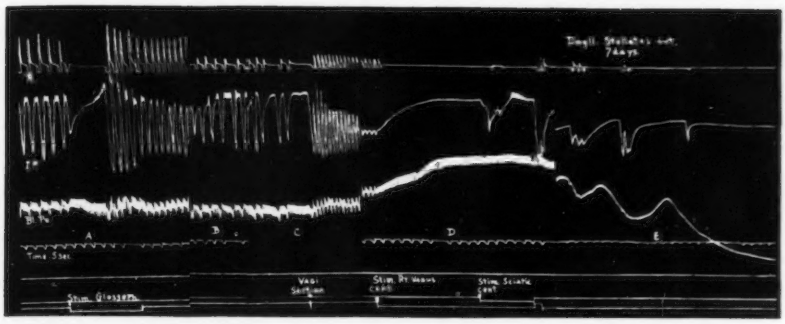

Fig. 2. Dog 11 in which the stellate ganglia had been removed seven days previously. Barbital anesthesia, 0.25 gram per kilo. *R*, respiration, tracheal; *I. P.*, intrathoracic pressure; *Bl. Pr.*, blood pressure, 136 mm. Hg. Section A of tracing shows response to central stimulation of glossopharyngeal. Section B, note the type of respiration after manipulation and ligation of the sciatic nerve, vagi having been dissected out previously; section C, vagi sectioned; section D of the tracing shows the effect of continuous stimulation of central end of right vagus until death resulted in section E of tracing. As indicated in section D, the sciatic was stimulated and some respiration occurred, which may happen spontaneously, however. The dog died eight minutes after beginning stimulation of central vagus (Harvard inductorium, 2 dry cells, secondary 6 cm.).

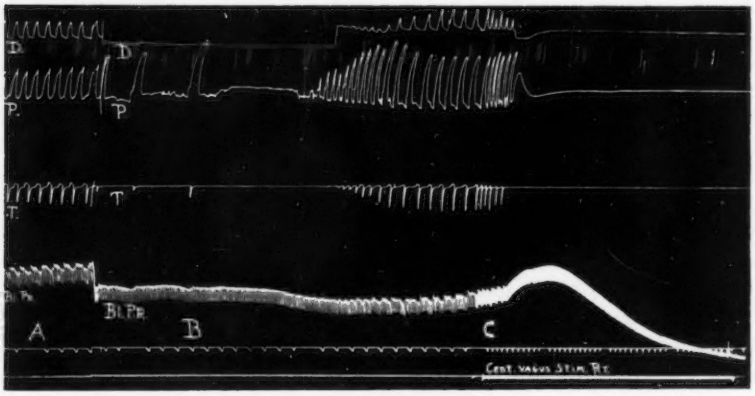

Fig. 3. Dog 80. In this animal, the stellates had been removed eight days before. *D*, diaphragmatic movements; *P*, pneumograph or chest movements; *T*, tracheal air exchange; *Bl. Pr.*, blood pressure. Tracing A shows the control, the blood pressure being 120 mm. Hg. Tracing B was taken while the vagi were being dissected and sectioned. Note the inhibition of respiration with recovery. The blood pressure fell to 84 as a result of this procedure. At the point of the tracing labelled C, the central end of the right vagus was stimulated (Harvard inductorium, coil 4 cm.) with a fatal inhibition of the respiratory center. Note that the rate of speed of drum was decreased at C. In B also note the disproportion between the chest diaphragmatic excursion.

tral vagi were stimulated in these dogs after removal of the stellates, less current was required for respiratory inhibition and there was less tendency for an occasional respiratory movement to break through. In two of these dogs, removal of one stellate was adequate to produce respiratory death on stimulation of the central vagi. On the basis of the analysis of the blood pressure records of this group, we believe that the low blood pressure incident to the more extensive operative procedures was a contributory factor.

Group V. In this group (6 dogs) we placed those experiments in which the current was applied high up on the vagus in the region of the ganglion nodosum and in which some spread of the current to the medulla may have occurred, or the respiratory inhibition may have been due to the stimulation of other nervous structures than the vagi. However, in no experiment did a spread of current occur sufficiently to excite the limb muscles, which should have occurred if there had been a direct spread of current to the medulla. Nevertheless, the results in this group are questionable because an occasional dog with stellates intact may die an asphyxial death on bilateral stimulation of the nerve trunks close to the jugular foramen.

One dog, in which the stellates had been removed four months previously, survived twenty minutes of bilateral stimulation of the nerve trunks in the region of the jugular foramen. During this twenty minute period, abdominal or diaphragmatic movements although very irregular in occurrence, were quite strong and effective in exchanging air. During the brief periods of apnea, shallow facial and abdominal respiratory movements appeared simultaneously, which increased gradually in depth and when reaching a maximum, a chest inspiratory movement occurred. After this twenty minute period of stimulation, respiration and blood pressure returned to normal. Then bilateral stimulation (full coil) of the nodose ganglia produced a respiratory death in eight minutes. Another animal in which the stellates had been removed one week before, withstood twenty minutes of stimulation of the vagi, after which normal respiration was resumed. Later the vagi were dissected out to the nodose ganglia. The dog died after eight minutes of stimulation of the nodose ganglia and associated nerves.

Respiratory changes. The effect of stellectomy on the rate, amplitude, and pattern of the respiratory excursion will be summarized in a subsequent paper when records will have been made on a larger group of animals. None of the stellectomized dogs died until the vagi were mechanically or electrically stimulated. The death was respiratory in type. Respiration generally ceased with the thorax in the expiratory position; but as the apnea continued, the side of the thorax would slowly increase in most dogs until a respiratory movement would break through. If the stimulus was applied initially at the peak of inspiration, expiration in a few instances was gradual, requiring ten seconds or more for completion. When respirations broke through in spite of continuous stimulation, they were preceded usually by movements of abdomen and mouth.

While the stellates were intact, respiratory escapement from vagal inhibition was prompt and accompanied usually by an increase in amplitude. After removal of the stellates, the recovery, after a brief period of central stimulation of the vagus, was sometimes more slow and gradual in regard to both rate and amplitude than prior to stellectomy. From one-half to three minutes or more elapsed before a maximum air exchange was manifest. During the final stimulus, when respiration would break through, the pneumograph would show occasionally much greater changes in the size of the chest wall and the intrathoracic pressure would show proportionately greater changes than air exchange. In other words, a marked respiratory effort would result in only a small amount of air exchange due probably to constricted bronchioles; or since it did not occur regularly, to an absence of coördination between the action of the bronchial muscles and the muscles of inspiration.

The intrathoracic pressure presented many interesting variations. Partial pneumothorax occurred in only two animals in this series. Removal of the stellates had a variable effect on the intrathoracic pressure; it usually became slightly less negative. The opposite was generally true of section of the vagi. These results might be expected to occur on the basis of changes in the tone of the diaphragm and the bronchial muscles after section of the vagi and stellectomy. Head (1889) found that freezing the vagi in the rabbit increases diaphragmatic "tone" and results to be reported later by us show that stellectomy decreases the "tone" of the diaphragm.

Stimulation of the sciatic nerve lowered the inspiratory negative pressure in the thorax to the same extent in an intact animal as in one in which the vagi are sectioned and stellates removed. Some of our records showed decisively that the change in negative pressure as related to air exchange is not dependent entirely on or proportional to the change in the size of the chest wall, for frequently sciatic or vagus stimulation before or after stellate removal would lead to a marked disproportional change. In several dogs after stellectomy and during the stimulation of the vagus centrally, abdominal movements occurred with definite changes in intrathoracic pressure, but the pneumograph showed little or no excursion of the chest wall and the tracheal pressure record showed air exchange, indicating a dissociation of the chest and abdominal (probably diaphragmatic) respiratory movements. In such instances the respiratory picture was not unlike that observed after sectioning the spinal cord at the level of the seventh cervical segment. Terminal changes in intrathoracic pressure were observed in three dogs which could not be accounted for except by assuming the occurrence of marked broncho-constriction or change in lung volume. We hesitate to suggest an explanation until we learn more about the state of the bronchial musculature and diaphragm under the conditions of our experiments. Asphyxia is a very likely factor, since Dixon and Brodie (1903)

have shown that inhalation of CO_2 leads to a constriction of the bronchioles which is not altogether central in origin.

The central end of the sciatic nerve was stimulated at various stages during the respiratory inhibition caused by central vagus stimulation after removal of the stellates. It was found that stimulation of the sciatic during the first minute or two would cause respiration to break through, but not to a normal extent (fig. 2). Stimulation of the sciatic during the terminal period of fall in blood pressure which occurred after the asphyxial rise, did not cause respiration to break through. Artificial respiration during the latter stages would occasionally resuscitate the respiratory center. After the blood pressure fell to 20 mm. Hg, artificial respiration failed to resuscitate the respiratory center, except in one dog, in which epinephrine was used. Spontaneous recovery of the respiratory center occurred in one dog in group II, when the stimulation of the central vagus was withdrawn at the point at which the blood pressure had fallen to the prestimulation level after the asphyxial rise. This was tried in only one dog in group II and with two dogs in group III with similar results.

Cardio-vascular effects. Removal of the stellates caused no decided change in the heart rate regardless of whether the vagi had or had not been sectioned previously. The removal of the stellates was generally accompanied by a 10 to 40 mm. fall in blood pressure, which did not occur in all cases. This, we believe, was due more to the operative trauma than to any other factor, since dissection of the stellates prior to excision caused a fall in blood pressure which was not changed by actual removal of the stellates after the dissection. If the blood pressure was around 100 mm. Hg at the start, removal of the stellates produced less change in blood pressure. The average blood pressure after removal of the stellates and section of the vagi in group II was 83 mm. Hg, the minimum being 66 and the maximum 100 mm. Hg. In group III, the average was 116, the minimum 110, and the maximum 128. In group IV, the average was 83, the minimum 70, and the maximum 100. The time required for stellectomy is not a factor, since after practice both ganglia may be removed in ten minutes.

An analysis of the data on the relation of the blood pressure level, at the time the final stimulus was applied to the central vagi, to the ease with which a respiratory death was produced, shows that low blood pressure is a contributory factor. That the blood pressure level is not a decisive factor is indicated by the fact that seven dogs in groups II, III and IV after the removal of the stellates had a blood pressure of 100 mm. Hg or more and on stimulation of the central vagi, death occurred within 6.7 minutes.

Within a minute after the application of the final stimulus to the central vagus, the blood pressure begins to rise and manifests the changes typical of asphyxia. No exceptions occurred in this series. As a rule, the heart rate increases definitely until the height of the rise in blood pressure is

reached. In four dogs there was no increase in rate. Extra systoles occurred terminally in only one dog in this series. Electrocardiographic tracings were made on five dogs. No abnormalities were observed other than the heart block of terminal asphyxia.

Miscellaneous. "Airedale" and "Police" dogs were used almost entirely, because of their size which facilitates the operative procedure. It was observed that the Police dogs have slender stellates and appeared to be more resistant to the fatal inhibition of respiration, and that Airedales have larger, shorter and thicker stellates, and are less resistant.

The results of the groups of experiments in which the stellates were removed may be summarized in a different manner, showing that electrical stimulation of the vagi is not always necessary to produce a failure of the respiratory center and that some animals are more susceptible than others. On electrical stimulation of the central vagi, fatal inhibition of the respiratory center was obtained in four animals after the removal of only one stellate. In five animals after both stellates had been removed, the respiratory center failed during or soon after the dissection of the vagi. In four animals no evidence of any kind of a respiratory movement was observed after the application of the final electrical stimulus was applied to the vagi. In fourteen, either irregular or typical Cheyne-Stokes respiration occurred during the application of the final stimulus.

Section of the spinal cord at different levels: Lower thoracic level. In order to be more certain that trauma and low blood pressure were not primary factors in depressing the respiratory centers, we sectioned the spinal cord at the level of the tenth thoracic segment. This procedure decreased the blood pressure and added considerable tissue trauma. Four dogs were so prepared. It was found that in spite of a marked fall in blood pressure in each case, continuous stimulation of the vagi centrally did not cause a fatal inhibition of the respiratory center. In these dogs on removal of the stellate ganglia, fatal inhibition of the respiratory center was obtained.

At level of the 7th cervical. The cord was sectioned at the level of the 7th cervical in two dogs. This produced the usual fall in blood pressure. It also interrupted the impulses transmitted to the respiratory centers via the stellates and the proprioceptive impulses from the intercostal and abdominal muscles. In both of these dogs, stimulation of the central end of the vagi caused a fatal inhibition of the respiratory center, but without the usual asphyxial rise in blood pressure. The picture was like that described by Mann (1918) when the central vagi were stimulated with the animal very deeply anesthetized. It is significant that both these dogs after section of the cord showed marked inhibition of respiration during dissection of the vagi. In one, the inhibition of respiration was so marked during the manipulation of the vagi that we were forced to proceed with the dissection at intervals.

DISCUSSION. On reviewing the literature, we have not been able to find a report in which the central end of the vagus was stimulated in the stellectomized dog. This is because most of the investigators in this field have either worked on animals under artificial respiration or have not studied central vagal effects after removal of one or both stellates.

As a result of our experiments, we are convinced that removal of the stellate ganglia including the third to fifth thoracic sympathetic rami renders the respiratory center more susceptible to vagus inhibition, which is sufficient to make it possible by continuous stimulation of the central vagi to produce an asphyxial death in most dogs. Low blood pressure is a contributory factor; but death is not due primarily to a cardio-circulatory failure as was observed by Mann (1918) to occur on central stimulation of the vagi in dogs so deeply etherized that the excitatory effect of central sciatic stimulation on the respiratory center was abolished. Our dogs responded normally to sciatic stimulation. Various strengths of stimuli were not used to detect a quantitative change.

The most likely explanation that we have to offer for this phenomenon is that the withdrawal of impulses transmitted by the stellate ganglia either depresses, "shocks," or disorganizes the activity of the central respiratory mechanism. Macklin (1929) has suggested that sensory impulses arising from the contracting bronchial muscles during expiration, pass to the medulla either by way of the vagi or sympathetics, to inhibit a hypothetical broncho-constrictor center which causes bronchial relaxation preparatory for inspiration. It is possible that in the dog such impulses pass via the stellate ganglia to the inspiratory center and excite it. The withdrawal of these impulses by stellectomy, then, would depress the tone of the inspiratory center and augment the inhibitory action of the vagus. Withdrawal of these impulses would also lead to a disturbance of the coördination that may exist between the movements of the bronchial muscles and the extrinsic muscles of respiration and account for the lack of correlation we observed between the inspiratory decrease in intrathoracic pressure and the air exchange in some animals. This view is supported by the experiments of Barry (1912–1913) on rabbits and cats which indicate that a collapsed condition of the lung and chest gives rise to sensory inspiratory impulses, the impulses passing to the spinal cord from the lungs via the stellates and from the chest muscles via the dorsal roots. The observations of Coombs and Pike (1930) and Gault and Scott (1918) who found in adult cats that division of the dorsal thoracic or cervical nerve roots depresses respiratory movements, agree with this view. The work of Craigie, who studied the the effect on respiration of irritating gases applied to the respiratory epithelium of dogs, indicates that the stellates mediate sensory impulses from the the lungs which affect respiration. In the rabbit, this does not appear to be true from the work of Larsell and Burget (1924), a point, however, on which

there is considerable disagreement in the literature. In this connection it should be pointed out that on anatomic grounds, there appears to be a greater sensory innervation of the lung in the dog than in the rabbit (Larsell and Burget, 1924).

Our observation that continuous vagal stimulation after stellectomy may produce a respiratory death, constitutes an additional example of a reflex fatal apnea to that which exists in the duck (Huxley, 1913; Koppanyi and Kleitman, 1927) in which animal continued dorsiflexion of the head will produce a fatal apnea, and shows that in the mammal strong nervous inhibition of the central respiratory mechanism may be under certain conditions sufficient to counteract the stimulating action of carbon dioxide.

It is at least evident from the results that after bilateral stellectomy, the added insult to the respiratory mechanism of continuous electrical stimulation of the vagi centrally and in some instances the mere mechanical manipulation of the vagi, is sufficient to produce a death that is predominantly respiratory in type. Further work will be necessary to elucidate the source and nature of the impulses transmitted by the stellate ganglia which antagonize the inhibitory action of the vagi on the respiratory centers.

SUMMARY

Removal of the stellate ganglia sensitizes the central respiratory mechanism to vagus inhibition. After removal of the stellate ganglia it is possible to produce a respiratory death by continuous strong stimulation of the central vagi, a result that is but rarely obtained in dogs with intact stellates unless the anesthesia is very deep (Mann, 1918). Low blood pressure is a contributory but not a primary factor. The stellate ganglia mediate impulses of functional importance in the regulation of the central respiratory mechanism.

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ON THE EXISTENCE OF AFFERENT RESPIRATORY IMPULSES MEDIATED BY THE STELLATE GANGLIA

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Because the experiments of Cromer and Ivy (1933) strongly indicate that the stellate ganglia mediate sensory impulses which affect the activity of the respiratory centers, it seemed desirable to repeat the work of those investigators who have studied this problem and have reported varying results. Barry (1913) clamped the trachea of rabbits at the end of inspiration and expiration and recorded the movements of the diaphragm before and after stellectomy. He concluded that sensory inspiratory impulses passed to the medulla via the stellates, while sensory inhibitory (expiratory-exciting) impulses passed over the vagi. Craigie (1922), using dogs, induced respiratory and circulatory changes by the introduction of irritating vapors (ammonia and ether) into the lungs, and concluded that the afferent impulses concerned traveled over the sympathetics to the cord and medulla, since sectioning of the vagi did not abolish the effect. Larsell and Burget, using a technique similar to Craigie's on rabbits and a few dogs, concluded that the afferent impulses concerned were mediated by the vagi alone in the rabbit and materially affect the induced respiratory reflexes in the dog, which agrees with the report of Mayer, Magne and Plantefol (1920), but not Roger (1917). However, Barry is the only investigator who actually removed the stellate ganglia in his experiments.

In this paper we shall report our results on the rôle played by the stellate ganglia and vagi in the reflex production of respiratory and circulatory changes induced by irritating vapors (ammonia) in the lungs.

METHODS. Dogs weighing from 18 to 25 kgm. were used. They were anesthetized with sodium barbital (250 mgm. per kilo body weight). Graphic records of femoral arterial blood pressure, chest movements (pneumograph) and intra-tracheal pressure were made. Ammonia was used as the irritating vapor, since ether was found in preliminary experiments to give less definite results. Air saturated with ammonia was obtained by bubbling air through a 40 per cent solution of ammonia. It was introduced into the trachea through a "16" gauge hypodermic needle inserted about three inches above the bifurcation of the trachea. The dura-

tion of injection of the ammonia vapor in each case was 5 seconds, i.e., the amount (approximately 10 mgm.) and duration of the "ammonia vapor stimulus" used was constant. Air introduced in a similar manner produced no effect on respiration. The experiments using ammonia vapor as a stimulus were carried out under the following conditions: *a*, vagi and stellates intact (10 dogs); *b*, vagi cut and stellates intact (6 dogs); *c*, stellates out and vagi intact (4 dogs); *d*, both stellates out and vagi sectioned (9 dogs).

The stellate ganglia and sympathetic chain were removed down to the third or fourth thoracic rami through a supraclavicular approach. The completeness of the removal was checked in each dog by post-mortem examination. Pneumothorax was ruled out by observing the respiration; and after the experiment was completed by exposing the parietal pleura between the ribs and noting that the lungs were in contact. With proper technique, the stellates may be removed routinely without puncturing the pleura and the muscles brought together with towel clamps to support the cervical pleura to prevent abnormal changes in negative intra-thoracic pressure incident to respiration.

RESULTS. *Chemical ammonia stimulation of the lower respiratory passages. Intact animals (10 dogs):* On injection of the ammonia vapor with all nerves intact the initial response in three dogs was a deeper inspiration followed by a deeper rather sudden expiration ("bechic blast" of Larsell and Burget, also Roger). An apnea varying from 5 to 33 seconds occurred in all but one dog. In this dog (dog 50), the amplitude of the respiratory movements was only slightly decreased. In the other dog (dog 39) not included in this series because he had pneumonia, the amplitude and rate of the respiratory movements were markedly diminished, but a period of apnea did not result. The period of apnea was followed usually by a period of faster and deeper respiration. The latent period of the response was never longer than five seconds.

Vagi cut, stellates intact (6 dogs): When the vagi were cut first, apnea did not occur on injection of the ammonia vapor. However, the respiration was decreased in rate and amplitude (fig. 1).

Stellates out, vagi intact (4 dogs): When the stellates were removed first, an apnea of six seconds resulted from the injection of the ammonia vapor in only one dog (fig. 2) in which an apnea of thirty-three seconds occurred before the removal of the stellates. In none of the other stellectomized dogs did injection of the ammonia vapor produce apnea, although an average apnea of 11 seconds had been obtained in all dogs with the stellates intact. However, a temporary reduction in thoracic excursions and air exchange occurred (fig. 2, section B).

Stellates out and vagi cut (9 dogs): After the removal of the stellates and section of the vagi, very slight or no change in respiration resulted on the

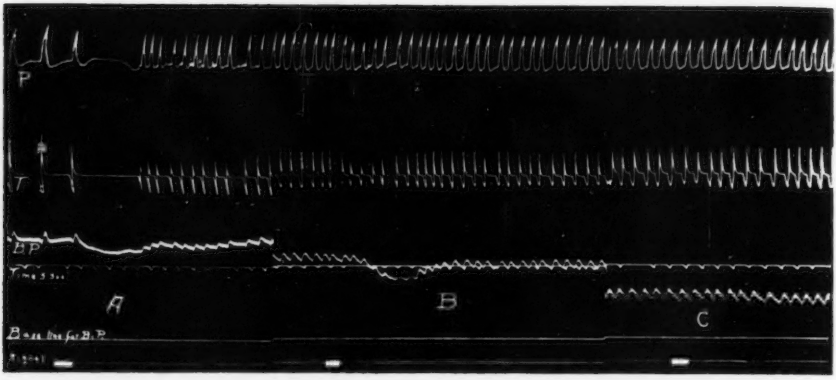

Fig. 1. Dog 49. *P*, pneumograph tracing (up is inspiration); *T* is tracheal air exchange (down is inspiration); *B.P.*, blood pressure. Section A shows the response of the intact animal to ammonia insufflation. Between sections A and B the vagi were cut, and between B and C the stellates were removed. Section B shows that an effect was still obtained after vagi section; and section C that all effects were abolished by combined vagi section and stellectomy.

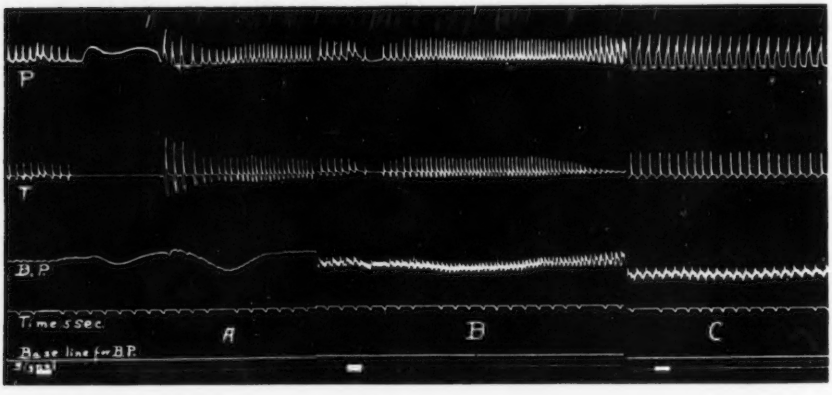

Fig. 2. Dog 41. This figure is labelled in the same manner as figure 1. Section A of the tracing shows the response of the intact animal to ammonia insufflation. Between sections A and B the stellate ganglia were removed. Section B shows the response to ammonia after stellectomy. Note at the end of section B the phenomena we interpret as being due to broncho-constriction. Between section B and C the vagi were sectioned. Section C shows that blood pressure and respiration were now unaffected by ammonia insufflation.

injection of the ammonia vapor. Slowing of repiration did not occur in any experiment. In two dogs a very slight decrease in amplitude of the thoracic excursion and air exchange resulted.

Other respiratory effects: As stated above, the initial deep inspiration and expiration ("bechic blast") described by other observers occurred in only three of our dogs (fig. 3). In one of the three dogs, this phenomenon did not occur after section of the vagi with stellates intact; in a second dog, it did not occur after stellectomy with the vagi intact; in the third dog, it occurred after section of the vagi and did not occur after subsequent stellectomy. Our explanation of this variation is that either vagi section or stellectomy may depress the respiratory center so that the impulses

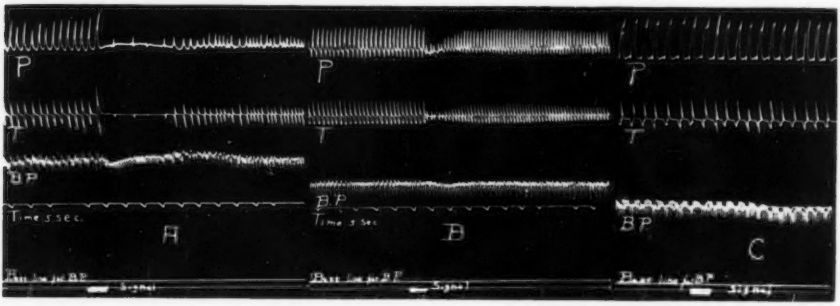

Fig. 3. Dog 51. This figure is labelled in the same manner as figure 1. In section A the ammonia insufflation caused in this animal a deeper inspiration followed by a deeper expiration. This was observed in only three of ten dogs. In the other dogs the expiration was much deeper. Between sections A and B the stellates were removed. In section B the deep inspiration and expiration did not appear on ammonia insufflation and the respiratory response was decreased and the blood pressure change was abolished by stellectomy. Between sections B and C the vagi were cut. No effect is now produced by ammonia insufflation.

concerned, which may pass either via the stellates or vagi, do not excite the respiratory center. Either this explanation is correct, or the impulses concerned pass via the stellates in some dogs and via the vagi in others.

In the intact animal after the initial response (apnea) to ammonia vapor, there is a gradual increase in rate, lasting for several minutes. This increase in rate is especially noticeable if the pre-insufflation rate is slow. Either vagotomy or stellectomy reduced this increase in rate, while combined vagotomy and stellectomy abolished it.

In four dogs with the nerves intact a definite reduction of air exchange resulted from one and one-half to two minutes after recovery from the apnea produced by the ammonia insufflation. In one instance only was

the decrease in air exchange associated with a decrease in chest excursion. The phenomenon is shown in figures 1, 2 and 3 toward the end of section B. This diminished air exchange lasted about one minute. Then the respiratory exchange gradually increased until it returned to normal in from three to five minutes. This phenomenon was manifested very definitely by all animals in which the stellates were removed first. (Study fig. 2, noting the end of the section of tracing labeled *B*.) In the animals in which the vagi were sectioned first (fig. 1), this phenomenon, due apparently to bronchial constriction, was not so evident or marked. It did occur in some of the dogs with both the stellates removed and the vagi sectioned, but to a less degree, however, than under the conditions mentioned.

Blood pressure changes: The blood pressure was affected in all dogs, but the type of response varied. As a rule it fell within ten or fifteen seconds after the injection of the ammonia vapor. The fall in pressure was followed by a gradual return to normal. A slight rise in blood pressure occasionally preceded the fall. In confirmation of Craigie, the circulatory response was practically abolished by stellectomy and little affected by double vagotomy. Heart rate was not affected markedly (figs. 1, 2 and 3).

Effect of ammonium chloride injected into the carotid artery: Even though it did not appear likely that the results we were observing following ammonia insufflation were due to absorption of ammonia into the blood, it was thought to be advisable to inject ammonium chloride into the carotid artery and ascertain its effect.

In our experiments on ammonia insufflation, approximately 10 mgm. of ammonia gas were introduced, which is equivalent to 31.5 mgm. of ammonium chloride. Of course it is very unlikely that all the ammonia was absorbed. When 100 or 200 mgm. of ammonium chloride were injected into the carotid artery during a period of five seconds (25–30 kilo dogs anesthetized with barbital), it was found that respiration was augmented for one or two respirations, then depressed slightly during several respirations. The blood pressure fell, the heart rate was decreased and the pulse pressure increased. This response was enhanced when 500 mgm. were injected. A marked diminution in respiration resulted with an apnea of *thirty* seconds in one dog. Although the picture obtained from the injection of ammonium chloride is analogous in some respects to the picture obtained with ammonia insufflation, the two pictures are not similar. Whereas vagi section and stellectomy markedly modify or abolish the response to ammonia insufflation, these procedures do not abolish or markedly modify the response to ammonium chloride injection. After combined vagi section and stellectomy ammonium chloride still caused a fall in blood pressure and a depression of respiration followed by stimulation. So we are certain that the responses observed on ammonia insufflation, as employed in our experiments, were of a true reflex nature.

Discussion. Our results confirm those of Craigie to the extent that some of the inhibition of respiration which occurs on the injection of ammonia vapor into the trachea is due to the integrity of the stellate ganglia, but we cannot confirm him in the statement that double vagotomy is without effect, which we do not think his own tracings show.

In our experience sectioning of the vagi does materially affect the deep respiratory reflexes elicted by chemical stimulation as is maintained by Larsell and Burget. However, we do not agree with their general conclusion, namely, that the afferent impulses concerned are mediated by the vagi alone. This may be true for the rabbit, but it is not true for the dog. The sectioning of the vagi did not entirely abolish the immediate response to ammonia vapor injected in the lungs, while the subsequent removal of the stellates did. The apnea was abolished by vagotomy (stellates intact) and also by stellectomy (vagi intact), the one exception being dog 41, in which stellectomy reduced the apneic period from 33 seconds to 6 seconds, as shown in figure 2. In animals with both the vagi cut and the stellates removed, there was no immediate response to irritation of the lungs with ammonia vapor. The response in regard to rate and amplitude was affected to about the same extent by stellectomy as by vagotomy. After section of the vagi and excision of the stellates, all responses initiated by the ammonia vapor injected into the trachea disappear, except that in which broncho-constriction may possibly be concerned.

The phenomenon which we ascribe to broncho-constriction is a decrease in air exchange which results in from 1 to 1½ minute after ammonia insufflation, and which occurs without a proportional decrease in chest excursion. In fact the amplitude of the chest excursion may be increased. This broncho-constriction, if it is a reflex, has an unusually long reaction time, but other reflexes are known which have a relatively long reaction time (e.g., the postural labrinthine reflexes described by Magnus (1924)). The long latent period may indicate that it required this length of time for the ammonia vapor to penetrate to and directly irritate the bronchial musculature. The broncho-constriction, if it may be called such, occurred frequently in the intact animal and was augmented by stellectomy but not by vagotomy. This may be expected on the basis of the sympathetics being broncho-dilator and the vagi broncho-constrictor nerves. However, the phenomenon still occurred although to a lessened degree, after section of the vagi and removal of the stellates which indicates the involvement of a local pulmonary mechanism in part. That a local broncho-constrictor mechanism may exist is indicated by the work of Dixon and Brodie (1903) on the broncho-constrictor action of CO_2 inhalation. Since post-mortem examinations showed an increase in mucus and fluid in the larger air passages, it might be true that the delayed decrease in air exchange is due to this and a posssible edema or congestion produced by the ammonia. Sev-

eral points oppose this hypothesis. First, since the increase in mucus and fluid formed is probably no greater in a vagotomized than a stellectomized animal, the amount of air exchange should not be varied more by stellectomy than by vagotomy. Second, the amount of exudate necessary to decrease air exchange would not likely be reabsorbed in a few minutes; nor would the amplitude of air exchange, especially after repeated stimuli, return to an original amplitude, if the exudate were drained to dependent portions or larger air passages. Third, if edema of the epithelium were an important factor in producing the variations, the air exchange should not return to normal level in three to five minutes.

It should be pointed out that associated with the operative removal of the stellate ganglia trauma is inflicted and that it is practically impossible because of anatomical relations to remove the stellates without at the same time possibly traumatizing the vagi, regardless of the operative skill developed and the method of approach. However, the tissue trauma inflicted by stellectomy is no greater than that inflicted by bilateral dissection of several inches of the vagi, and the vagi are more traumatized by dissection than they are by stellectomy. That these unavoidable factors do not enter to question the significant feature of our results is shown by the fact that all the immediate effects of ammonia stimulation were not abolished until the stellates were removed and the vagi sectioned.

As stated before, the responses observed are not due to the distention of the lungs since the same quantity of air was inert; neither can they be explained as due to the absorption of ammonia.

Our results on dogs indicate that on stimulation of the lower respiratory passages with ammonia vapor, both excitatory and inhibitory impulses may pass over the vagi and stellates to the respiratory center. In our experiments, the inhibitory effect predominated. In this connection it is of interest to point out that Boothby and Berry (1915) observed apnea on distention of the lungs in two dogs in which the pulmonary branches of both vagi had been sectioned three months previously and under conditions which led them to believe that the apnea was not chemical in type. We do not believe that we have sufficient evidence to state that the impulses mediated by the stellate ganglia are specific in character, that is, that they are inspiratory-inhibiting or stimulating, or expiratory-inhibiting or stimulating. But we believe that it is clear from our observations that the respiratory center may be inhibited by impulses mediated by the stellate ganglia, and that in experiments dealing with the nervous control of respiration as influenced by stimuli acting in the lungs, the stellate ganglia as a pathway cannot be overlooked.

SUMMARY AND CONCLUSIONS

Afferent impulses initiated by the insufflation of ammonia (approximately 10 mgm.) vapor into the trachea, which influence respiration, are

mediated by both vagi and stellate ganglia. The predominant effect as observed in our experiments is inhibition of respiration which is definitely reduced by bilateral excision of the stellate ganglia or section of the vagi. Combined section of the vagi and bilateral excision of the stellate ganglia abolish all respiratory effects except a decrease in air exchange which occurs from 1 to 1½ minute after the insufflation of the ammonia. This decrease in air exchange is interpreted as being due to broncho-constriction. The decrease in air exchange is more evident after stellectomy with the vagi intact than under other conditions. The circulatory effect of the ammonia insufflation, which is variable, a fall in blood pressure resulting usually, is practically abolished by bilateral stellectomy, and is little affected by bilateral vagotomy. The stellate ganglia as a sensory pathway for central reflex effects on respiration and circulation cannot be disregarded in experiments on the dog in which the sensory nerve endings in the lungs are stimulated.

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OSMOTIC RELATIONS BETWEEN BLOOD AND BODY FLUIDS

IV. Pancreatic Juice, Bile and Lymph

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Although the chemical ingredients of the alimentary secretions have long since been defined, it is only recently that the physico-chemical implications of the concentration of their constituents have received attention. Gamble and McIver (1928) noted that the total anion content of the gastric juice and the total cation content of the pancreatic juice and bile were such as to make them approximately isotonic with the blood. This would imply that whereas the alimentary glands function in such a manner as to produce secretions of a characteristic chemical composition, it is probably by a physico-chemical equilibration with the blood that the concentration of the ingredients of the respective secretions is defined.

Previous papers in this series (Gilman and Cowgill, 1931, 1933) have reported studies indicating that the concentration of ions in the gastric juice elaborated by the parietal cells is determined by the osmotic pressure of the blood, the two fluids being isotonic. Any variation in blood osmotic pressure is accompanied by parallel changes in the composition and osmotic pressure of the gastric secretion. The present paper is a report of similar experiments on pancreatic juice and hepatic bile. In addition osmotic pressure determinations were made on the lymph.

Experimental procedure. Five acute experiments were conducted. Dogs, anesthetized with amytal, were used as subjects. The secretions under examination were obtained by cannulation of the pancreatic duct, the common bile duct after ligating the cystic duct, and the thoracic duct. The flow of pancreatic juice was stimulated by the intravenous injection of secretin prior to obtaining a sample. Normal samples of these fluids were obtained simultaneously with blood from the femoral artery. The animals then received intravenously 5 cc. per kilogram of 10 per cent NaCl. After 30 minutes, samples were again collected. The subjects then received intravenously 75 cc. per kilogram of distilled water.

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The water was added slowly over a period of 90 minutes to reduce hemolysis as much as possible. Thirty minutes after the completion of the water injection a final group of samples was obtained. In one experiment the procedure was reversed, water being injected before the hypertonic saline. Osmotic pressure determinations were made immediately after the completion of each experiment. The method of Hill (1930) was employed.

TABLE 1

Changes in osmotic pressure of pancreatic juice, bile and lymph accompanying osmotic changes in the blood

EXPERIMENT	PROCEDURE	BLOOD	LYMPH	BILE	PANCREATIC JUICE
		m.eq.	*m.eq.*	*m.eq.*	*m.eq.*
I	Normal	156	154	150	154
	Salt	162	162	156	160
	H_2O	155	157	146	144
II	Normal	155		148	147
	Salt	174		168	172
	H_2O	142		138	139
III	Normal	159	159	154	155
	Salt	175	176	169	168
	H_2O	146	145	144	143
IV	Normal	156	155	152	146
	Salt	165	165	157	158
	H_2O	145	144	146	141
V	Normal	157	157	151	149
	H_2O	135	134	133	140
	Salt	147	148	148	144
Average		155	155	151	151

Osmotic pressure is expressed as milli-equivalents of an osmotically equal sodium chloride solution.

RESULTS. The experimental results are summarized in table 1. The approximate isotonicity between the blood and the fluids under examination is at once apparent. Changes in the osmotic pressure of circulating fluid in either direction were accompanied by parallel changes in the osmotic pressure of the pancreatic juice and bile. Also the blood and lymph were at all times isotonic.

DISCUSSION. The implication of these results is evident. It would seem obvious that the primary factor concerned in defining the total concentration of the elements of the bile and pancreatic juice is the osmotic

pressure of the blood. Thus the secretions that enter directly into the gastro-intestinal tract in the largest volume, namely, gastric juice, bile and pancreatic juice, are already isotonic in composition. Saliva is the one exception to the isotonicity of the alimentary secretions. Measurements made upon the osmotic pressure of parotid saliva, and mixed secretion of the sublingual and submaxillary glands (unpublished data) show both of these fluids to be isotonic with a NaCl solution approximately 40 M. Eq. in strength.

The impression is also gained that gall-bladder bile is likewise anisotonic because of its high Na content (Gamble and McIver, 1928). Recent investigations on the equilibration of salt solutions placed in the gall bladder show that such fluids tend to become isotonic with the blood (Ravdin, Johnston, Austin and Riegel, 1932). Measurements made upon the osmotic pressure of bile found in the gall bladder show its freezing point to be approximately the same as blood (Ravdin, Johnston, Riegel and Wright, 1932). It would seem that although the gall-bladder bile is concentrated with respect to the proportion of Na and water, the greater part of this base must be in an unionized form with the result that this fluid, despite its high solid content is approximately isotonic with the blood. Thus with the exception of the saliva, every digestive fluid which enters the gastro-intestinal tract in appreciable quantities is of such a composition as to help maintain the isotonicity of the intestinal contents.

As would be expected, lymph and blood are practically identical with respect to their osmotic pressures. These data answer certain criticisms which have been directed against the Hill method for the determination of vapor pressure. The method depends upon a measurement of the rate of evaporation from the surface of the fluid being tested. It has been claimed that in certain biological materials the formation of a film on the surface of the fluid being measured would interfere with the estimation of its vapor pressure. In this study osmotic pressure determinations were made on defibrinated whole blood. That this fluid containing corpuscular elements and an appreciable quantity of protein does not tend to form any surface film which would tend to impede its rate of evaporation is shown by the following facts: 1, its vapor pressure determined by the Hill method is practically identical with that of lymph, a fluid containing less protein and few cellular elements that would be expected to have little or no tendency to form a surface film and 2, estimation of the concentration of osmotically active ions in the blood by physical measurement with the Hill method checks perfectly the total concentration of ions found by chemical analysis. The authors are confident that the values for osmotic pressure presented in this paper are accurate to within 1 M. Eq., when expressed as concentration of an osmotically equivalent NaCl solution.

SUMMARY AND CONCLUSIONS

Simultaneous collections were made of dogs' blood, hepatic bile, pancreatic juice and lymph. Measurement of the osmotic pressure of these fluids, using the Hill method, showed them to be practically isotonic. Extreme variations in the osmotic pressure of the blood, produced by the administration of hypertonic saline or water, resulted in parallel changes in the osmotic pressure of the above fluids. It is believed that whereas the alimentary glands are unique in the production of their characteristic secretions, the *total concentration* of their constituents is determined by a process in common, namely, osmotic equilibration with the circulating fluid.

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THE EFFECT OF MOVING LIQUID UPON THE ELECTRICAL STIMULATION OF MUSCLE

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It is recognized that the tone of plain muscle is maintained with the utilization of very small amounts of oxidizable materials and with the production of very little heat (1). The present investigation was begun originally to determine if plain muscle, immersed in a weak sugar solution, would use the sugar, and if electrical stimulation and oxygen gas would influence the rate of this utilization. It was soon found that the bubbling of oxygen through the sugar solution in which the muscle was suspended increased the height of the muscular contractions immediately, and if the plain muscle was contracting rhythmically it increased the height and rate of these contractions. At first this was thought to be due to the beneficial effect of the oxygen. However, after it was found that other gases, such as nitrogen, hydrogen, and carbon dioxide, produced the same effect, this view was abandoned and a systematic study was undertaken to determine the difference, if any, in effect of O_2, N_2, H_2, and CO_2 on the contractions of skeletal, cardiac and plain muscle when bubbled through the surrounding liquid.

The following is a description of the method employed in making this study. One end of a gastrocnemius muscle of a frog was attached to a platinum hook fused in the bottom of a glass tube 1 cm. in diameter and 10 cm. long and the other end was attached to a lever. After the glass tube had been filled with Ringer's solution, the apparatus was arranged so that the muscle could be stimulated once every second with different strengths of induction shocks, using an electrical clock for making and breaking the primary circuit. Gas cylinders were also arranged so that the different gases could be bubbled at the rate of 4 cc. per second through the Ringer's solution in the tube in which the muscle was suspended. In figure 1, tracing a, it will be seen that the muscle was first stimulated with sub-minimal induction shocks and no contractions were produced. The oxygen was then bubbled through the Ringer's solution, in the tube around the muscle, while the sub-minimal stimulation was continued. Each time it caused the sub-minimal stimuli to produce almost maximal contractions. The sub-minimal stimulation was then discontinued and the oxygen run in and no

contractions were produced. Nitrogen, hydrogen and carbon dioxide were then used in the same way that the oxygen had been and it was found that these gases produced the same effect as the oxygen, that is, they increased the effectiveness of the sub-minimal stimuli, causing them to produce almost maximal contractions, while the gases themselves without the electrical stimuli produced no contractions.

In figure 1, tracing b, is shown the effect of O_2, N_2, H_2, and CO_2 on muscular contractions when sub-maximal stimuli were used. It will be seen that these gases increased also the effectiveness of the sub-maximal stimuli. In tracing c is shown the effect of the gases when the muscle was stimulated with maximal electrical stimuli. Under this condition the gases had no effect on the height of contractions. It should be stated in this connection that the same gastrocnemius muscle was used in making all the tracings

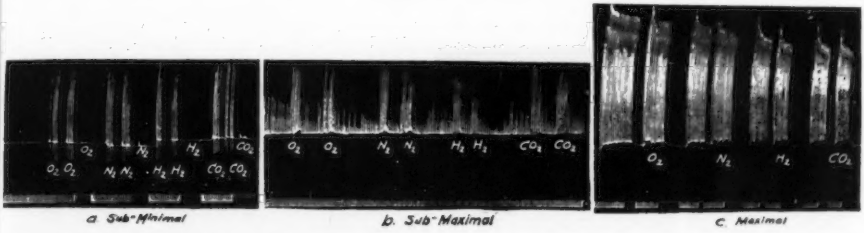

Fig. 1. Tracings showing the effect produced on the height of contractions of skeletal muscle when stimulated with sub-minimal, sub-maximal, and maximal induction shocks, by bubbling oxygen, nitrogen, hydrogen, and carbon dioxide through Ringer's solution in a tube in which the muscle was suspended.

Tracing a shows that the gases increased the effectiveness of sub-minimal electrical stimuli causing them to produce almost maximal contractions although the gases when used alone were ineffective as stimuli.

Tracing b shows that the gases also increased the effectiveness of sub-maximal electrical stimuli, thereby producing higher contractions.

Tracing c shows that the gases had no effect on the height of contraction of the muscle when maximal electrical stimuli were used.

shown in figure 1. Many experiments similar to the preceding were carried out with comparable results.

Similar experiments were carried out on muscles from curarized frogs. In figure 2 is shown the effect produced on the contractions of a frog's curarized gastrocnemius muscle when stimulated with sub-minimal, minimal, sub-maximal, and maximal induction shocks. Here again the bubbling of the gas caused sub-minimal, minimal, and sub-maximal stimuli to produce practically maximal contractions while it had no effect on the height of the contractions when maximal stimuli were used. Whatever effect is produced by the gas is exerted directly on the muscular tissue.

Fig. 2. Tracings showing that the bubbling of O_2 through a tube containing Ringer's solution, in which was suspended a curarized frog's gastrocnemius caused subminimal stimuli to produce practically maximal contractions, and increased the effectiveness of minimal and submaximal stimuli, but had no effect on the height of the contractions when maximal stimuli were used.

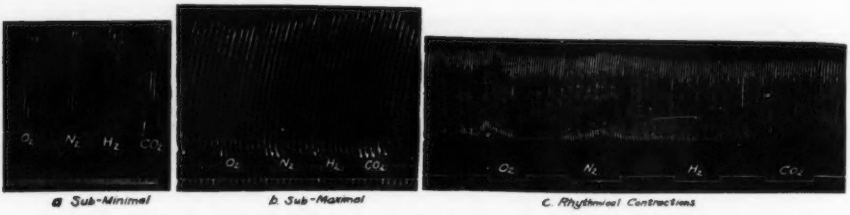

Fig. 3. Tracings showing the effect produced on the height of contractions of cardiac muscle when stimulated with sub-minimal and sub-maximal induction shocks by bubbling oxygen, nitrogen, hydrogen and carbon dioxide through Ringer's solution in a tube in which the muscle was suspended.

Tracing a shows that the gases caused sub-minimal electrical stimuli to produce maximal contractions of cardiac muscle.

Tracing b shows that the gases had no effect on the contraction of cardiac muscle when sub-maximal electrical stimuli were used.

Tracing c shows that the gases had no effect on a rhythmically contracting heart.

Fig. 4. Tracings showing the effect produced on the rhythmical contractions of plain muscle by bubbling oxygen through the Ringer's solution in the tube in which the muscle was susended as well as the effect of circulating the Ringer's solution.

Tracing a shows that oxygen increased the height and rate of the rhythmical contractions of plain muscle.

Tracing b shows that circulating Ringer's solution around the muscle also increased the height and rate of the rhythmical contractions of plain muscle.

Our next experiments were carried out on cardiac muscle. A strip of the turtle's ventricle was suspended in the tube in Ringer's solution and stimulated in the same way that the frog's gastrocnemius had been. In figure 3 is shown the effect of the different gases on the height of contractions of cardiac muscle when stimulated with sub-minimal and sub-maximal electrical stimuli. Tracing a shows that the gases caused sub-minimal stimuli to produce maximal contractions of the heart muscle. Tracing b shows that sub-maximal electrical stimuli produced maximal contractions in keeping with the "all or none" law and that the bubbling of the gases produced no further increase in the height of the contractions. The same strip of ventricle was used in making both of the preceding tracings.

Tracing c, figure 3, shows that the bubbling of the gases through the Ringer's solution in the tube in which was suspended a rhythmically beating turtle's heart had no effect on either the height or rate of the rhythmical contractions.

Plain muscle was used in the next experiments. A piece of the turtle's pylorus was suspended in the glass tube in the Ringer's solution in the same way as had been the cardiac and skeletal muscle. Tracing a, figure 4, shows that the bubbling of oxygen through the Ringer's solution in the tube in which was suspended the piece of rhythmically contracting plain muscle increased the height and rate of the contractions. It will be recalled that the gases had no effect on either the height or rate of the rhythmically contracting heart muscle and hence cardiac and plain muscle differ in this respect.

In tracing b, figure 4, is shown the effect of circulating Ringer's solution around a piece of rhythmically contracting plain muscle suspended in the glass tube. The circulating Ringer's solution increased the height and the rate of the rhythmically contracting plain muscle in the same way as did the bubbling of oxygen.

SUMMARY

1. Bubbling various gases through the bottle of Ringer's solution in which a skeletal muscle is suspended increases the effectiveness of sub-minimal and sub-maximal electrical stimuli.

2. In the case of strips of heart muscle under similar conditions, ineffective stimuli are converted to effective stimuli.

3. With rhythmically contracting strips of plain muscle, bubbling gas through the solution or simply circulating the solution causes an increase in the height and rate of the contractions.

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STUDIES IN THE PHYSIOLOGY OF VITAMINS

XXI. The Effect of Water Administration on the Development of Anorexia and Polyneuritic Symptoms in Pigeons Subsisting on a Diet Lacking the Vitamin B Complex

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One of the theories as to the cause of the neuromuscular manifestations of lack of antineuritic vitamin B attributes these phenomena to the presence of a toxin. The remarkably prompt relief of these symptoms obtained by administration of the vitamin has led many investigators to favor the toxemia idea. Cowgill, Rosenberg and Rogoff (1930) tested this hypothesis in dogs and came to the conclusion that administration of large amounts of fluids by mouth to dogs subsisting on a diet relatively free from undifferentiated vitamin B markedly shortens the time required for the appearance of the anorexia characteristic of lack of this dietary factor. In view of the fact that much of the experimental work on vitamin B has been done on birds, it was deemed of interest to repeat the experiment on the pigeon and to determine, if possible, whether forced administration of fluids to this species would significantly protract or diminish the period of time required for the development of both the anorexia and the neuritic symptoms characteristic of lack of antineuritic vitamin.

The only experimental work bearing directly on this question, that we have been able to find, is that of Eijkman and von Hoogenhuyze (1916) and Marrian and co-workers (1927), the results of which are contradictory. The former investigators used fowls from which all food was withheld and reported that during such a starvation régime the outbreak of the neuritic symptoms characteristic of vitamin B deficiency was then hastened by "washing out" the birds with water. They concluded that their results did not support the toxin theory. On the other hand, Marrian and associates reported that in their experiments on birds, whose diet was free from the antineuritic vitamin, there was just as much tendency for convulsions to occur in pigeons whose consumption of water was limited to the

[1] This is a report of some of the data contained in the thesis submitted by M. L. Palmieri to the faculty of the Yale University School of Medicine, April, 1932, in partial fulfillment of the requirements for the degree of Doctor of Medicine.

very small quantity that they drank voluntarily as in birds receiving a daily administration of large volumes of water. A contributory fact of interest concerning the fluid balance of the body in vitamin B deficiency is that discovered by Rose, Stucky and Mendel (1930) who found that a variable degree of anhydremia occurs in this condition and that the voluntary fluid intake of the experimental animals (dogs) is diminished when the antineuritic factor is absent. As regards the physiological rôle of body fluids, Underhill and Kapsinow (1924) stated their belief that a great factor of safety exists with respect to the maintenance of the water balance of the body.

EXPERIMENTAL PART. *Plan of experiment.* One of the characteristic features of vitamin B deficiency with which this experiment is concerned is anorexia. The simplest and most dependable method for determining the onset of this important symptom consists in the observation of the daily body weight changes. Hoet (1923) has shown that the loss of appetite in pigeons subsisting on a diet deficient in the antineuritic vitamin is quite prompt in nearly every instance, and that the appetite can therefore be followed either by measuring the amount of food eaten voluntarily or by observing the variations in the body weight curve. Other investigators have confirmed this observation. Klotz (1926) used the latter technique and found it very satisfactory for pigeon experiments. Illustrations of the use of this method are given elsewhere (Block, Cowgill and Klotz, 1932; Cowgill, Deuel, Smith, Klotz and Beard, 1932) and therefore need not be presented here. In the first half of this experiment the pigeons received, in addition to the usual allotment of vitamin B-deficient food and water, two 45 cc. doses of water administered daily by way of the crop. As soon as any bird developed the typical neuritic symptoms, the water administration was stopped and large doses of a fuller's earth absorbate of vitamin B[2] were given. After suitable realimentation the experiment was repeated but with the forced water administration omitted.

Diet used and other experimental details. The bulk of the calories was supplied by polished rice allowed *ad libitum*. This, however, is not a completely adequate food (McCollum and Davis, 1913; McCollum and Simmonds, 1917; Chick and Hume, 1917–18), because it is low in protein and salts and is practically devoid of known vitamins. For completely satisfactory experiments it is necessary that these essentials be supplied. The birds, therefore, received daily administrations of commercial meat residue, which has been found to lack vitamin B (Osborne and Mendel, 1917; Cowgill, Deuel and Smith, 1925); the salts were furnished by the Osborne-Mendel (1917) salt mixture. Vitamins A and D were supplied in the form of cod liver oil. Details with respect to quantities used,

[2] Kindly furnished by Dr. C. N. Frey of the Fleischmann Research Laboratories, New York City.

method of administration, and discussion of the adequacy of this ration for the pigeon are presented elsewhere (Block, Cowgill and Klotz, 1932).

Eight pigeons of mixed varieties were used at the beginning of the study. Two birds served as controls and received as addition to the basal ration adequate amounts of the fuller's earth adsorbate of vitamin B prepared from yeast. Three of this first group of birds failed to recover from the crisis of extreme vitamin B deficiency and were replaced by new birds for the test during the second period when forced water administration was omitted. These new birds were not put through a period of water administration because the results obtained at that time were so concordant that it seemed unnecessary. The effect of the previous nutritive history on the time required for the development of anorexia was controlled by administration of large amounts of the vitamin B concentrate to each bird at the beginning of the experiment in order to "saturate the tissues" with vitamin B. The above described plan was followed rather than to have the two groups running simultaneously because previous studies of anorexia in this laboratory indicate the importance of using a given animal as its own control.

Results—Discussion. The data are summarized in the accompanying table.

It appears that under the conditions of our experiments, the administration of large volumes of fluid by mouth to pigeons subsisting on a diet deficient in the vitamin B complex was without appreciable influence on the length of the period required for the development of the typical anorexia.

The appearance of neuritic symptoms indicative of advanced vitamin B_1 deficiency in pigeons was delayed slightly when large volumes of water were given. The question arises as to whether the slight difference here noted is significant. Withington (1900) studied the effect of administration of moderate amounts of fluids to starving dogs and concluded that water administration to a fasting animal materially diminishes the loss of body weight and prolongs life to a corresponding degree. Inasmuch as the pigeons in this experiment were on practically a starvation régime, the difference in the durations of the period prior to the development of neuritic symptoms may perhaps be explained on this basis. It seems reasonable, therefore, to conclude that the difference here observed is without significance.

These results confirm those of Marrian and associates rather than the findings of Eijkman and von Hoogenhuyze. They do not agree with those obtained by Cowgill, Rosenberg and Rogoff (1930) on the dog. No satisfactory reason for this is apparent. It is possible that in the dog the diuresis, which resulted from the administration of water, caused a washing out of vitamin B from the organism. In the bird, however, the elimina-

tion of nitrogen through the kidney involves urates chiefly, which remove less water; furthermore, the urine is voided into the cloaca in common with the intestinal excreta, and it is conceivable that this allows for some reabsorption of previously "washed out" vitamin. If these suggestions are correct, then it would be difficult indeed to obtain in the bird results similar to those obtained by Cowgill, Rosenberg and Rogoff on the dog.

TABLE 1

PIGEON	DAYS REQUIRED FOR THE DEVELOPMENT OF			
	Anorexia with		Neuritic symptoms with	
	Fluids forced	Fluids not forced	Fluids forced	Fluids not forced
A (control)	No anorexia	No anorexia	No symptoms	No symptoms
B (control)	No anorexia	No anorexia	No symptoms	No symptoms
G	8	8	34	28
C	8		51 (died)	
F	9		46 (died)	
E	9		43	
D	11	12	39	32
H	11	9	34	20
I		6		44 (died)
K		7		28
J		8		44 (died)
Average.....	9 ±1.0	8 ±1.3	38 ±3.5	27 ±3.5

CONCLUSIONS

The administration of large volumes of water by mouth to pigeons subsisting on a diet deficient in the antineuritic vitamin is without appreciable influence on the duration of the period required for the development of anorexia due to lack of vitamin B.

The appearance of neuritic symptoms typical of advanced vitamin B deficiency in pigeons is delayed slightly when large volumes of water are administered. This delay is so slight, however, that it is probably without significance.

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EFFECT OF CORTICAL HORMONE IN PREVENTING EXTREME DROP IN COLONIC TEMPERATURE DISPLAYED BY HYPOPHYSECTOMIZED RATS UPON EXPOSURE TO COLD WITH PRELIMINARY OBSERVATIONS UPON THE EFFECT OF HYPOPHYSEAL AND OTHER HORMONES[1]

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Investigators who study hypophysectomized rats soon discover that such animals have a very low tolerance for cold and run a subnormal rectal temperature. Similarly, adrenalectomized rats are very sensitive to cold temperatures, and Hartman (1931) has shown that when adrenalectomized rats are placed out-doors on a cold winter day they will undergo a pronounced drop in colonic temperature. He was able to protect his adrenalectomized rats from this fall in temperature by preliminary injections of Cortin, the hormone of the adrenal cortex. As a clinical parallel to this effect of Cortin in adrenalectomized rats, Baird and Albright (1932) in a study of four cases of Addison's disease, noted that Cortin therapy caused a disappearance of the tendency of such patients to show a subnormal rectal temperature.

The extreme atrophy of the adrenal cortex seen after the removal of the hypophysis cerebri would lead one to anticipate that hypophysectomized rats might show, when exposed to low temperatures, a drop in colonic temperature comparable to that seen in Hartman's adrenalectomized rats. One might likewise wonder whether Cortin would protect hypophysectomized rats as it does adrenalectomized rats. In this study it has been shown that both these forecasts hold true.

Removal of the hypophysis results in atrophy not only of the adrenal cortex but of almost all of the other glands of the endocrine system. The adrenal medulla is a notable exception (Smith, 1930). The thyroids, testicles, and ovaries all undergo easily demonstrable shrinkage in size. The testicles of normal rats become so small and so retracted that they cannot be delineated upon palpation. Functionally, this atrophy is prominently

[1] The expenses of the investigation were defrayed by a grant from the Delamar Mobile Research Fund.

[2] Medical Resident, 1930–1932.

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illustrated in the female by the disappearance of the oestrous cycle. It is clear that a lessened function of any or of all of these glands might contribute to the loss of resistance to cold temperatures which occurs following hypophysectomy.

In order to study the possible contributory effects of thyroids, testes and ovaries, observations were made upon thyroidectomized, castrated, and ovariectomized rats, and these observations were compared with similar observations on adrenalectomized as well as hypophysectomized rats. The effects of thyroxin and theelin administration on hypophysectomized rats were likewise investigated.

For the purpose of analyzing the specificity of Cortin in protecting against a drop in temperature on exposure to cold, control determinations were done with injections of normal salt solution and 5 per cent glucose and the effect of starvation was determined in one rat.

Finally, a few additional experiments were performed to determine the loss of which of the several hormones of the anterior pituitary was responsible for the loss of resistance to cold. These experiments included the injection of the following substances into hypophysectomized rats: 1. Pituitary growth and gonad-stimulating hormone (extract of anterior lobe, Parke, Davis and Company).[4] 2. Pituitary thyrotropic hormone.[5] 3. Gonad-stimulating hormone of urine of pregnant women—Antophysin (Winthrop Chemical Company[6]). 4. Pituitrin

Technique of hypophysectomy. The operation of hypophysectomy was carried out in this study by a method very similar to the parapharyngeal approach described by P. E. Smith (1930) and K. W. Thompson (1932). Ether anesthesia was used. The floor of the pituitary fossa was exposed and a small piece of bone was removed by trephining in an area located by landmarks pointed out to us through the instruction of Dr. K. W. Thompson. These landmarks have been clearly described in the papers by Smith and Thompson already referred to. Upon removal of the trephine a brisk hemorrhage often occurred but this was controlled quickly by adrenalin packs. The pituitary gland lying in its sheath could be seen clearly. By means of a small glass cannula connected with a water-suction pump, the pituitary (anterior and posterior lobes) was evacuated quickly and usually completely. Careful search was then made for any pituitary remnants which might have clung to the circular shelf of bone left on the ventral surface of the pituitary fossa. As pointed out by

[4] The authors take this opportunity of thanking Parke, Davis & Company for supplying this hormone.

[5] This hormone was furnished by the Medical Research Division of the Schering Corporation. Dr. Gregory Stragnell in a communication to one of the authors stated that this preparation was perfected by Prof. Schittenhelm of Kiel. The term "thyrotropic" was first used by F. A. E. Crew and W. P. Wiesner, (1930) of Edinburgh in which he described a separate hormone of the anterior pituitary which stimulates the thyroid.

[6] The authors take this opportunity of thanking the Winthrop Chemical Company for supplying this hormone.

P. E. Smith (1930), the diaphragma sellae of the rat is a very firm and tough membrane and is left uninjured by hypophysectomy carried out by his technique. Because of this feature, rats can be hypophysectomized with very little danger of injury to the brain, and it is unlikely that such injury plays a part in the picture of the hypophysectomized rat. Ether anesthesia was found to be highly satisfactory and was used in most cases. "Dial" anesthesia was much less serviceable in our hands than was ether. From ether anesthesia our rats recovered very quickly and they could clear their air passages of the mucoid secretions collecting there during the operation. The longer period of unconsciousness present after the use of "Dial" seemed to increase the mortality markedly.

EXPERIMENTAL PROCEDURE. The colonic temperatures and weights of a given group of albino rats under observation were taken at room temperature. These rats were then placed in separate jars containing no food or water and were placed in a cold room at a fairly constant temperature varying from one experiment to the next from 0.0°C. and 5.5°C. Separate jars were used in order to prevent the variable effect of huddling together. Subsequent colonic temperatures were then taken at varying intervals until tolerances were determined. The records taken were compared with previous records on the same rat and with records on other rats exposed to the same temperature in the same experiment.

As often as possible a rat was used as its own control. Thus, when Cortin protection was being studied, alternating experiments with saline and with Cortin were carried out. Two weeks were allowed for the effect of Cortin to wear off.

Rats which showed a great drop in temperature were placed after removal from the cold room in clean, dry jars with food and water and allowed to recover slowly. The use of an external source of heat to bring about a rapid rise in temperature seemed to act very unfavorably and several rats died following this treatment. Others recovered from even lower temperatures if they were simply restored to room temperature and allowed to recover slowly.

At the conclusion of this investigation, all rats were chloroformed and carefully autopsied. Serial sections of the pituitary fossae were made to determine the completeness of the operation of hypophysectomy.

For determining the colonic temperatures a narrow Centigrade thermometer was lubricated with mineral oil and then inserted a distance of six to eight centimeters and held there for two minutes after the mercury column had seemed to stop moving. After being in the cold room a few hours, most of the rats would develop a diarrhea which would leave the large intestine open so that the thermometer could more easily be inserted. In most instances, the thermometer could be inserted to the level of the liver. Temperatures so taken were termed colonic rather than rectal, in agreement with Hartman's (1931) suggestion.

RESULTS. *A. Normal, hypophysectomized, and adrenalectomized rats.*

1. *Normal rats.* (See table 1.) The average cold room temperature employed for testing animals was 3.6°F. At this temperature, the average hypophysectomized rat in five hours underwent an average drop of 11.5°C. (20.7°F.) in colonic temperature. Normal rats were allowed to remain in the cold rooms, at the same average temperature, for an average of 14.5 hours and in this time they underwent an average drop of only 1.4°C. Some of the normal rats stayed in the cold room from twenty-two to twenty-four hours and showed a fall of less than 1°C. The limit of tolerance for normal rats was not determined in any instance.

2. *Hypophysectomized rats.* Hypophysectomized rats, on the other hand, showed an abrupt decline in body temperature as great as 21°C. (38°F.) in less than three hours (see table 1). No rat was intentionally allowed to undergo a drop of more than 10°C. The very low temperatures recorded took place in instances wherein unpredictably great drops in

TABLE 1

Comparison of normal rats and hypophysectomized rats

Average figures

	AVERAGE TEMPERATURE IN COLD ROOM	AVERAGE COLONIC TEMPERATURE		AVERAGE FALL IN TEMPERATURE	AVERAGE NUMBER OF HOURS IN COLD ROOM	REMARKS
		Start	Finish			
	°C.	°C.	°C.	°C.		
Normal	3.8	38.4	37.0	1.4	14.5	15 observations on 11 normal rats
Hypophysectomized	3.3	37.7	26.3	11.5	5.3	25 observations on 13 hypophysectomized rats

temperature occurred in intervals between thermometer readings. It was to our utmost astonishment that hypophysectomized rats recovered from temperatures as low as 16°C. (60.8°F.) and lived in good condition thereafter for weeks, surviving several similar subsequent experiments.

3. *Adrenalectomized rats.* Two bilaterally adrenalectomized rats were studied. As compared with the average hypophysectomized rats, these rats showed the same sharp drop in temperature in less than half the time. These adrenalectomized rats showed the same lack of resistance to cold temperatures two days after adrenalectomy as they showed at six days following the operation. One died eleven days and the other died fourteen days following adrenalectomy.

B. *Effect of Cortin and adrenalin on hypophysectomized rats.* 1. *Effect of Cortin.* Cortin was used in six experiments on five rats. Control observations were made in each case before and after Cortin protection experiments, and in all instances the resistance of hypophysectomized rats

to cold temperatures was increased very markedly. With some lots of Cortin, larger doses were required to produce the effect than with others.

In table 2 are recorded average figures which showed the effect of Cortin. The five rats used were tested at an average temperature of 3°C. The average cold room temperature for experiments before, during and after

TABLE 2

Effect of Cortin on resistance of five hypophysectomized rats to cold

Average figures. Cold room temperature 3°C.

	FALL IN COLONIC TEMPERATURE	NUMBER OF HOURS IN COLD ROOM
	°C.	
15 days before Cortin experiment	12.1	5.8
During Cortin treatment	3.9	11.4
15 days after Cortin experiment	11.9	4.8

TABLE 3

An experiment with two pituitarectomized rats and a normal control

RAT	DATE	PRELIMINARY TREATMENT	TEMPERATURE IN COLD ROOM	HOURS IN COLD ROOM	FALL OF COLONIC TEMPERATURE	REMARKS
			°C.		°C.	
Experiment 1						
Hypophy. R. T.	6–1–32	Cortin	0.5	8	2.8	6 cc. Cortin in 1 cc. doses
Hypophy. R. P.	6–1–32	Saline	0.5	8	17.6	6 cc. normal saline in 1 cc. doses
Normal	6–1–32	Saline	0.5	8	0.0	6 cc. normal saline in 1 cc. doses
Experiment 2						
Hypophy. R. T.	6–16–32	Saline	2.2	6	13.2	35 cc. normal saline in 5 cc. doses
Hypophy. R. P.	6–16–32	Cortin	2.2	9	1.9	35 cc. Cortin in 5 cc. doses
Normal	6–16–32	Saline	2.2	9	0.6	35 cc. normal saline in 5 cc. doses

Cortin injections fluctuated only slightly. The resistance of hypophysectomized rats was increased on the average of about six-fold (roughly estimated), taking into consideration the lessened fall in colonic temperature and the increased period of exposure to cold.

In table 3 are recorded the results of two consecutive experiments in

which two hypophysectomized rats and one normal rat were compared. It will be seen that subcutaneous injections of Cortin raised the resistance of the hypophysectomized rat, R. T., toward normal in the first experiment, in which R. T. showed a drop of only 2.8°C. in eight hours. Two weeks later, after the effect of Cortin had worn off, this same rat underwent a drop of 13.2°C. in six hours. The reverse procedure was carried out in the case of hypophysectomized rat, R. P. In the first experiment, R. P. received preliminary injections of saline, and showed a drop of 17.6°C.

TABLE 4

Data obtained from pituitarectomized rat, R. P.

DATE	PRELIMINARY TREATMENT	TEMPERATURE OF COLD ROOM	COLONIC TEMPERATURE		FALL IN TEMPERATURE	HOURS IN COLD ROOM	WEIGHT		REMARKS
			Start	Close			Start	Close	
		°C.	°C.	°C.			*grams*	*grams*	
6- 1-32	Saline	0.5	37.6	20	17.6	8			Testicles not palpable
6-16-32	Cortin	2.2	38.4	36.5	1.9	9			35 cc. Cortin in 5 cc. doses
6-23-32	Saline	2.2	37.5	27.2	10.3	8			35 cc. saline in 5 cc. doses
7- 1-32	None	4.4	38.2	26.5	11.7	7¾			
7-19-32	Cortin	5.0	38.2	37.3	0.9	13¼			30 cc. Cortin in divided doses
8- 6-32	Saline	2.2	38.2	25	13.2	3	134	131	25 cc. saline in divided doses
8-21-32	Prolan	2.2	37.7	25.2	12.5	3½	128	124	250 units "Antophysin" (Winthrop)
8-27-32	Pituitary growth hormone	2.7	37.4	24.5	12.9	3	138	136	12 cc. Ant. Lobe Ext. P. D. & Co.
8-31-32	None	2.7	37.2	25.3	11.9	2	137	129	
8-31-32	Adrenalin	2.7	37.6	23.8	13.8	2½	136	132	0.4 cc. of 1 to 10,000 adrenalin in 2 doses

in eight hours. Two weeks later, after preliminary injections of Cortin, this rat stayed in the cold room nine hours with a drop of only 1.9°C. in temperature.

In table 4 are shown the results of successive experiments with rat, R. P. Saline injections were used alternately with Cortin injections, and tests were carried out at intervals of seven to eighteen days.

Reasoning from the premise that atrophy of the adrenals would account for the lowered resistance to cold temperatures seen in hypophysectomized

rats, one might anticipate that days or weeks would be required for the necessary atrophy to develop. This, however, was not the case. Removal of the pituitary was followed immediately by a depression of the resistance to cold temperatures.

Measurements made at intervals soon after hypophysectomy indicated that the effect developed before atrophy could take place. Observations made at longer intervals following hypophysectomy indicated that as atrophy developed the effect grew only slightly greater. The resistance at 16, 17, 18 and 23 hours following hypophysectomy was approximately the same as that found at 38, 64, 68, and 164 days following hypophysectomy. Rat 15, when tested at three days after operation, showed a drop of 10°C. in four hours. This same rat, when tested at thirty days, showed a drop of 12°C. in 3.8 hours. Rat 4, at seventeen hours after operation, showed a drop of 12.5 °C. in seven hours, at thirty-eight days after operation, a drop of 12.9°C. in 4.5 hours.

All rats that were tested in less than twenty-four hours after the operation were in excellent condition; they were very lively and manifested no evidence whatsoever of any abnormality. Nevertheless, it might be argued that the effect found was a post-operative phenomenon. In order to test this supposition, a rat was subjected to all the usual operative procedures, including the removal of a plate of bone from the floor of the sella turcica, but leaving the pituitary intact. This rat, twenty-six hours later, withstood a temperature of 2.2°C. for twenty-five hours with a drop in temperature of only 1.3°C. In contrast to this control animal, hypophysectomized rat 12, when tested at twenty-three hours following the operation, showed a drop of 12.8°C. in five hours in a cold room at 3.3°C.

2. *Effect of adrenalin.* Adrenalin, in doses of 0.2 cc. of 1 to 100,000 solution and 0.4 cc. of 1 to 10,000 solution showed no influence whatsoever on the resistance of two hypophysectomized rats to cold temperatures. Similarly, Hartman (1931) found adrenalin ineffective in adrenalectomized animals.

C. *Results of experiments related to other glands markedly affected by hypophysectomy.* 1. *Effect of thyroxin administration.* In one instance it was demonstrated that thyroxin in a toxic dose (2 mgm.) brought about a very great increase in the resistance of an hypophysectomized rat to cold temperatures. In this instance, R. T., after saline injections showed a drop of 13.2°C. in six hours at 2.2°C. Four days after receiving 2 mgm. of thyroxin, this rat showed a drop of only 3.5°C. in eight hours at 2.2°C. In this case, protection by thyroxin was only slightly less than the protection given by Cortin injections in a previous test on the same rat. Nine days after receiving thyroxin, R. T. was quite strikingly emaciated, but, when tested, showed excellent resistance to the cold up to seven hours. In seven hours, the temperature fell only 5.7°C., as compared with 13.2°C.

in six hours during the previous test after saline injections. However, during the next two hours, R. T. showed a further sharp drop of 13.1°C. One might conjecture that R. T. showed good resistance on thyroxin in the second experiment, as long as its glycogen storage held out, but when this storage was exhausted, its temperature fell rapidly.

In a second experiment, hypophysectomized rat 3 showed a slightly increased resistance after subcutaneous injections of two less toxic doses of thyroxin (two doses of 0.5 mgm. each). With no preliminary treatment, rat 3 showed a drop of 10°C. in five hours at 3.8°C. Four days later, after receiving thyroxin, this rat endured the same temperature for the same time with a drop of only 6.9°C.

2. *Resistance of thyroidectomized rats.* Because of this surprising influence of thyroxin, it seemed to be a matter of much interest to study the effect of total thyroidectomy on the resistance of rats to cold temperatures. Since thyroxin had been used in toxic rather than physiologic doses and since thyroxin had proved to be less effective in smaller than in enormous doses, we were not justified in anticipating how thyroidectomized rats would perform.

Dr. W. T. Salter of the Huntington Memorial Hospital very kindly supplied us with two thyroidectomized rats. Ten days following the operation of thyroidectomy, one of these rats showed a drop of 7°C. in eight hours at 2.7°C. These figures represented a resistance about twice as great as that seen in the average hypophysectomized rat. The other rat performed in a normal fashion, showing a drop of only 0.3°C. in ten hours at 2.7°C.

Twenty-six days following thyroidectomy, both of these thyroidectomized rats endured a cold room temperature of 2.7°C. for sixteen hours with a fall of only 3°C. in colonic temperature. Resistance as great as this was well within normal limits.

Metabolic proof of hypothyroidism we did not obtain on these rats and one rat died before developing clinical myxedema. The other, however, in the opinion of Doctor Salter, did become myxedematous.

3. *Resistance of ovariectomized rats.* Ovariectomized rat 1 showed the same remarkable resistance to cold temperatures seen in castrated male rats soon after operation. Sixteen hours after ovariectomy, this rat had shown a drop of only 0.6°C. in twenty-three hours at 3.8°C. Thirty-five days after ovariectomy, the resistance was still normal, but at forty-nine days the temperature fell 11°C. in ten hours at 2.2°C.

Ovariectomized rat 2 showed a prompt diminution in tolerance following ovariectomy, but was still about 100 per cent more resistant than the average hypophysectomized rat. At forty-two hours and at nine days following ovariectomy, this rat showed a reduced resistance almost identical with that seen in ovariectomized rat 1 at forty-nine days following the operation.

4. *Effect of theelin.* This experiment in a sense is the antithesis of the preceding except that ovariectomy constitutes the removal of the seat of production of two hormones—estrin and progestin—and in this experiment only one of these (estrin) was injected. Hypophysectomized female rat 15 received subcutaneous injections of 240 units of Theelin in divided doses and was kept in full oestrus for four days. Two experiments were carried out during this four-day period, and in both the resistance was found to be slightly less than that observed in the preceding experiment with saline injections.

5. *Resistance of castrated male rats.* Before castration, rat 1 resisted a temperature of 4.4°C. for twenty-four hours, with a drop of only 0.4°C. Three days after castration, this rat's resistance was just as good as before, and it endured a temperature of 3.8°C. for twenty-three and a half hours with a rise of 0.5°C. Sixteen days after castration, rat 1 still showed normal resistance. Forty-three days after castration, however, the tolerance was found to have fallen. After seven hours in the cold room at 1.6°C., this rat's temperature had dropped 3.5°C. but its resistance at this point was much better than the average hypophysectomized rat.

After continuing the experiment to eleven hours, the temperature fell 19.7°C. The rat recovered very satisfactorily and was tested again at 51 days, when a similar result was obtained.

Castrated rat 2 up to nine days following castration had a resistance wholly as good as before operation.

D. *Results of experiments with possible non-specific factors.* 1. *Normal salt solution.* In six experiments, the results obtained from saline injections were compared with the results seen in the same animals after no injections whatsoever. Saline produced no demonstrable effect.

2. *Glucose.* Following 30 cc. and 54 cc. subcutaneous injections of 5 per cent glucose, hypophysectomized rat 4 showed the same lack of resistance observed with saline injections and with no treatment at all.

3. *Starvation.* Although the experimental animals in this study did not show more than slight weight variations during the time intervals involved in each series of experiments, we were nevertheless a little worried as to the possible effect of incidental variations in the caloric intake. Because of this, a starvation experiment was performed as follows: A female rat with a low normal resistance to cold showed a drop of 5.6°C. in ten hours at 1.6°C. This rat was then starved for forty-one hours and its weight fell from 172 grams to 152 grams. It was then tested and showed a better tolerance than before. At the same temperature and in the same time its body temperature fell only 2.8°C. From this experiment it was concluded that slight daily variations in the caloric intake of

experimental animals probably had very little influence on their resistance to cold temperatures.

E. *Effect of administration of pituitary extracts and prolan to hypophysectomized rats.* It was a point of great interest to determine which of the various hormones of the pituitary gland was capable of restoring to hypophysectomized rats their normal resistance to cold temperatures. In this report, only a few preliminary observations can be offered.

1. *Prolan.* Pregnancy urine extract (Antophysin, Winthrop Chemical Company) was given daily in subcutaneous doses of 50 units each for five days in two hypophysectomized rats. In both cases the increased susceptibility to cold was then found to be still present, as judged by previous experiments with and without saline injections. In both cases, Cortin had been used previously to produce very decided protection against cold temperatures.

2. *Growth hormone and gonad-stimulating hormone.* An alkaline extract of the anterior lobe of the pituitary containing both the growth hormone and the gonad-stimulating hormone (Parke, Davis & Company) was tested on three hypophysectomized rats. In two rats, a total of 18 cc. each was given subcutaneously in the course of seven daily injections. In a third rat, a total of 12 cc. was given in six daily doses of 2 cc. each. Tests then showed that the tolerances were still decreased and the figures obtained checked amazingly well with saline control studies on the same rat.

3. *Thyrotropic hormone.* The pituitary thyrotropic hormone was tested on two rats. In both cases very marked increase in tolerance for cold temperatures was demonstrated. Daily subcutaneous injections of 2.5 mgm. each were given for eight days in the case of hypophysectomized rat 14 and for seven days in the case of hypophysectomized rat designated S. H. R. In the case of S. H. R., the thyrotropic hormone proved to be exactly as efficacious as Cortin had been in a previous experiment, and both produced an approximately five-fold increase in the resistance to a temperature of 3.3°C. Rat 14 was also markedly protected by the thyrotropic hormone. At a cold room temperature of 2.7°C., rat 14 showed in a preliminary experiment a drop of 11.8°C. in seven hours. After receiving 20 mgm. of thyrotropic hormone, rat 14 at the same cold room temperature and in the same time, showed a drop of only 3.9°C. After stopping the injections, the resistance of rat 14 continued to rise and four days later this rat endured a temperature of 2.4°C. for twenty-three hours, with a drop of only 3.4°C.

4. *Pituitrin.* To rule out if possible the posterior pituitary as a factor, we tried twice on the same hypophysectomized rat to increase its tolerance with pituitrin. In one experiment, the rat which weighed 160 grams received 0.1 cc. of obstetrical pituitrin one-half hour before being put into the ice box; in the other experiment, it received 0.1 cc. at the time of being

put into the ice box and again one hour later. In both experiments the resistance to cold was even less than in two control experiments without pituitrin.

Autopsy findings. Since all hypophysectomized female rats showed total absence of the oestrous cycle, since all hypophysectomized male rats showed testicular atrophy which in time became so great that the testicles could not be clearly palpated, and since none of these rats gained in weight following hypophysectomy, it was fair to assume that at least a very considerable fraction of the pituitary had been removed in each case.

Autopsy studies, nevertheless, were directed chiefly toward a search for pituitary remnants by examination of serial sections of the sella turcica. In one instance (S. H. R.) the sella turcica was filled with bits of bone, fibrous tissue, blood-forming elements and one small island of cells indistinguishable from pituitary tissue. This rat showed a drop of 13°C. in temperature in three hours, when exposed to a cold room at 3.3°C. Its tolerance for cold temperatures was less than other rats in which no microscopic evidence of pituitary remnants was found. For this reason it seemed justifiable to assume that the small pituitary remnant seen in S. H. R. had negligible function.

In only one other case were there histologically demonstrable pituitary remnants and in this case the gland revealed by microscopic examination was fully as large as the usual normal pituitary body. This rat (L. H. R.) had no oestrous cycle as judged by repeated vaginal smears, but its resistance to cold temperatures in three experiments was quite normal, and in one experiment, this rat was left in the cold room at 5.5°C. for twenty-three hours and showed a drop of only 0.7°C. in colonic temperature.

Discussion. These experiments seem to present physiological evidence which may now be added to anatomical evidence (Smith, 1930) to show that the picture of pituitary insufficiency in rats in part at least is due to an associated adrenal cortex insufficiency and can be corrected by Cortin. They bring up the question whether the syndrome of pituitary cachexia of humans (Simmond's disease) may not be partly due to an associated hypocortinism, and whether Cortin therapy might not be indicated in such patients in the absence of a potent pituitary extract. In this connection it is of interest that Bauer (1927) makes the following statements regarding Simmond's disease in his text book: "The patients frequently complain of feeling cold and the body temperature as a rule sinks below normal. Often there exists a marked lowering of the arterial blood pressure, which makes one think at first of Addison's disease."[7]

The observation that loss of resistance to cold temperatures occurred in less than 24 hours after hypophysectomy was interpreted to mean that removal of the pituitary caused a very sharp decline in the function of the adrenal cortex. This interpretation is supported by the anatomical studies of P. E. Smith (1930), who showed that in six days following hypophysectomy the adrenals weighed only 50 per cent of the weight of controls. This extremely rapid atrophy of the adrenal glands was also shown by

[7] Translation is our own.

Smith to be due almost entirely to retrocession of the cortical portion of the adrenals. Our studies strongly indicated that atrophy of the adrenal cortex followed in the wake of diminished function and was possibly the delayed effect of withdrawing an anterior pituitary hormone which played directly and continuously upon the adrenal cortex and governed its size and function.

Gonadectomized rats and thyroidectomized rats behaved in a manner quantitatively very different from that seen in hypophysectomized and adrenalectomized rats. The comparative figures obtained placed hypophysectomized and adrenalectomized rats in a class together so far as tolerance for cold temperatures was concerned. The effect seen after gonadectomy required such a long latent period that we were inclined to conclude that ovaries and testicles *per se* had no effect on susceptibility to cold. Whether or not gonadectomy acted by promoting a slowly diminished function of the adrenals was not studied.

The rôle of the thyroid glands, as judged by three out of four observations on two thyroidectomized rats, seemed to be insignificant up to 26 days following thyroidectomy. After longer intervals, these rats might have shown diminished tolerance to cold temperatures as deduced from experiments of Hammett (1923) and of Herring (1920). In 1923, Hammett showed that, at 50 days following removal of the thyroid glands in rats, the adrenal glands underwent atrophy amounting to 14 per cent in males and 35 per cent in females. P. E. Smith has pointed out that this atrophy is less prompt and much less pronounced than that seen after hypophysectomy. In view of these considerations, any loss of resistance to cold temperatures, which might have developed at longer intervals after thyroidectomy, could be explained on a basis of atrophy of the adrenal glands.

It would be interesting to know the loss of which of the several hormones of the anterior pituitary is associated with decreased resistance to cold. Our experiments throw some light on this question. A preparation containing the growth hormone and the gonad-stimulating hormone did not restore this resistance to hypophysectomized animals. Likewise the gonad-stimulating hormone of pregnant urine was without effect. Considerable evidence has accrued, however, that this latter hormone is not identical with the gonad-stimulating hormone of the anterior pituitary (see Reichert et al., 1932). But the thyrotropic hormone of the anterior pituitary did restore this lost property to hypophysectomized rats. One would wonder whether it did so by stimulating their adrenals, but further work is necessary. Whichever hormone of the anterior pituitary it is which is concerned with this mechanism, the authors believe that there is now available an excellent measuring stick for detecting said hormone—namely, the hypophysectomized rat on exposure to cold.

CONCLUSIONS

1. Within 24 hours following hypophysectomy a great loss of tolerance for cold temperatures occurred in rats. This tolerance decreased only slightly in subsequent weeks.

2. At an average cold room temperature of 3.6°C. hypophysectomized rats in 5 hours underwent an average drop of 11.5°C. in colonic temperature. Normal rats could tolerate the same average temperature for an average of 14.5 hours with a drop of only 1.4°C. in colonic temperature.

3. Hypophysectomized rats recovered from colonic temperatures as low as 16°C. (60.8°F.).

4. Cortin almost completely restored the resistance of hypophysectomized rats to cold temperatures.

5. Thyroxin in toxic doses increased the tolerance of hypophysectomized rats to cold temperatures.

6. The thyrotropic hormone of the pituitary likewise almost restored the tolerance of hypophysectomized rats to cold temperatures.

7. Theelin, adrenalin, normal saline, 5 per cent glucose, the pituitary growth hormone together with the pituitary gonad-stimulating hormone, the gonad-stimulating hormone of pregnancy urine, and pituitrin did not influence the resistance of hypophysectomized rats to cold temperatures.

8. Gonadectomy was followed within two months by a diminution of resistance to cold temperatures.

9. Thyroidectomized rats were approximately as resistant as normal rats to cold temperatures up to 26 days following thyroidectomy.

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THE RATE OF ENTRANCE OF FLUID INTO THE BLOOD IN HEMORRHAGE

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The object of this series of investigations was to study quantitatively the redistribution of water between blood and tissues in response to various influences. The plan was to sample blood, and sometimes other tissues, at very brief and frequent intervals in anesthetized dogs that were subjected to acute procedures of several sorts. First the normal variations in concentration with time and manipulation were ascertained, second the influences on this concentration of rapid hemorrhage were studied. These two sets of results are recorded in the present paper.

Exchanges of water between the circulating blood and the spaces outside the blood vessels may be provisionally pictured as dependent chiefly upon two kinds of forces; hydrostatic pressure and effective osmotic pressure. The hydrostatic pressure that is operative is the difference between the mean pressure of the blood inside the capillaries in a particular mass of tissue and the opposing mean pressure of the extravascular extracellular fluids in the same tissue. The osmotic pressure that is effective is the difference between that exerted by constituents of the blood plasma and that exerted by constituents of the interstitial tissue fluids in the mass of tissue across the capillary blood vessel walls. According to the classic work of Starling (1896) the colloidal constituents of the plasma and of the tissue fluids, hence chiefly proteins, are the dissolved constituents to which the capillary endothelium is ordinarily little permeable; therefore they alone are able to exert osmotic pressure.

The ranges within which the whole blood or the plasma or the tissue mass may change their concentrations and volumes over periods of many hours are fairly well known. But very few and incomplete data are available to show how soon and how fast transfers of fluid may occur. It was the particular aim of the present experiments to measure in detail the amounts of fluid exchange taking place from minute to minute. For this purpose several kinds of analyses were made upon many samples of blood taken at close intervals. In view of the intensive character of the data, no great series of separate experiments was attempted.

PROCEDURES. All experiments were made upon dogs. Usually large

animals were chosen in order that the amounts of blood and other tissues drawn for analysis would be small compared to the total quantities present.

Two anesthetics were used, either sodium amytal or ethyl urethane, injected into the peritoneal cavity, in doses sufficient to suppress all reflex responses to cutting the skin. The effects of these anesthetics upon water distribution are dealt with in another paper (Adolph and Gerbasi, 1933); the initial changes were over and the blood had reached a relatively steady composition, characteristic for each anesthetic, before hemorrhage or other experimental procedure was begun.

Operative procedures involved the permanent occlusion of a certain number of blood vessels. From one of the carotid arteries, pressures were recorded by either a mercury manometer or a membrane manometer with a very light writing lever. The left brachial artery was clamped off and cut, and blood samples were collected from its central end. From the right jugular vein or femoral vein venous pressure was recorded, the cannula being inserted through the junction of a branch. Either a mercury manometer or a water manometer was used to record the venous pressure. The trachea was always cannulated.

Blood samples were run directly from the cut end of the artery into weighing bottles, which were immediately stoppered and placed in covered moist chambers. Ordinarily three samples of 1 to 2 cc. each were taken successively, just after the dead space of the artery had been allowed to flush out. The first of these samples was run into a bottle containing 0.2 to 0.5 mgm. of heparin and was thoroughly shaken. The other two samples were run into bottles whose tare weights were newly determined every time they were to be used.

Tissue samples were excised with scissors, working very quickly so that extraneous blood would not contaminate the samples. In all instances liver, and in some instances muscle, was tied off by strong ligatures just as excision occurred. Duplicate or triplicate samples were obtained from adjacent portions of the same piece of tissue.

Seven kinds of analyses were done upon blood samples, usually in duplicate or triplicate. Three of these related to the concentration of the plasma, two to that of the corpuscles, and two to that of whole blood. These were:

Refractive index of heparinized plasma (*PR*) by the dipping refractometer.

Specific gravity of plasma (*PG*) by the falling drop method of Barbour and Hamilton (1925).

Water content of plasma (*PW*) by drying weighed samples of 0.07 to 0.25 gram at 90° to 100°C. to constant weight.

Hematocrit (*BT*) by measuring the height of *red* corpuscles after centrifuging all samples simultaneously for 30 minutes at 2200 R.P.M. in thick-walled capillary tubes of 15 cm. length, the tops of which were 11 cm. from the axis of rotation.

Hemoglobin concentration of whole blood (*BH*) by diluting all samples alike, saturating with carbon monoxide, and reading in a colorimeter provided with a green Wratten filter no. 74.

Specific gravity of whole blood (*BG*) by the falling drop method.

Water content of whole blood (*BW*) by drying weighed samples of 0.6 to 2.0 gram at 90° to 100°C. to constant weight.

All the samples except those for water content were easily obtained from 1.5 cc. of heparinized blood, plasma analyses being made from the hematocrit samples.

Tissues were analysed in the present series of experiments only by evaporating weighed samples (0.5 to 2.0 gm.) at 100°C. to constant weight; or, where appreciable oxidation occurred in the samples, to minimal weight. The analyses of water content are designated according to whether muscle (*MW*), liver (*LW*), skin (*SW*) or intestine (*IW*) was sampled.

The object was to measure the *amounts* of water that were translocated by relating the quantities of fluid found in the samples to particular constituents of the blood or tissue that might be considered as constant or nearly constant in total amount present in the tissue. Hence all results were expressed as unit of liquid found per unit of solid constituent found, which is referred to as the dilution ratio. Such a method of calculation was also used by Robinson and Parsons (1931). Therefore: *PR* = (100 − per cent weight of protein) ÷ per cent weight of protein; the percentage of protein was computed from the refractive index by means of the tables of Reiss (1913); but the relative changes in *PR* were the same if computed by means of the formula of Neuhausen and Rioch (1923). *PG*, *BG* = 1 ÷ (specific gravity − 1). *PW*, *BW*, *MW*, *LW*, *SW*, *IW* = weight of water evaporated ÷ weight of dry residue. *BT* = (100 − per cent RBC volume) ÷ per cent RBC volume. *BH* = (100 − per cent weight of Hb) ÷ per cent weight of Hb. Since no absolute standard was used in the colorimetric comparison of hemoglobin concentration, it was assumed for the calculation that the basal or initial concentration of hemoglobin after anesthesia was in each amytalized dog 13 grams per 100 cc. of whole blood, and in each urethanized dog 15 grams per 100 cc. of whole blood.

In all cases the ratio of liquid to original wet weight, which is the more customary value, may be obtained from any dilution ratio given (r) by the relation $\frac{r}{1+r} \times 100$ = per cent water content of wet tissue. In none of the determinations are the absolute amounts of various constituents to be considered; for this reason the ratios found in the samples taken just before the experimental influence was introduced were taken as 100 per cent, and all previous and subsequent analyses were compared to these.

Controls. Three kinds of controls were required for proper evaluation of the data obtained on blood dilution. These were obtained by determin-

ing the mean deviations of 1, analyses of tissue from the same sample; 2, analyses of tissues taken at successive separate times, and 3, analyses of tissues whose excisions were separated by a time corresponding to the duration of a complete experiment (60 to 90 minutes). The first indicated the errors inherent in the methods; these errors decreased markedly during the months of experimentation and the figures to be given are based on results obtained in the last six months of a two-year period during which similar analyses were being done weekly. In these six months all analyses of any one kind were carried out by the same individual. The second kind of control showed the variations in the composition of tissues under the conditions prevailing in the collection of the samples. The third kind brought to light any time factors or trends in these variations due to expo-

TABLE 1

Percentage mean deviations in dilution among analyses and among samples of blood taken from operated dogs

	PR	*PG*	*PW*	*BT*	*BH*	*BG*	*BW*	*MW*	*LW*	*SW*	*IW*
1 A. Same sample kept in moist chamber 3 hours							−0 1	−0 05	−0 05	−0 05	−0 05
B. Duplicate analyses of same sample taken in control period	±0 4	±0 3	±2 0	±1 6	±2 3	±0 2	±0 4	±2 6	±1 6	±7 7	±2 9
C. Duplicate analyses of same sample taken 32 to 55 minutes after an infusion	±0 7			±0 9	±1 4	±0 2	±0 8	±5 3	±1 9		
2 A. Successive samples taken in control period	±0 9	±0 9	±2 0	±1 3	±1 2	±0 3	±0 8	±4 8	±2 8	±8 5	±4 6
B. Successive samples taken from symmetrical parts of body				±1 7			±0 8	±2 4			
3 A. Samples taken one hour apart, 2 hours after amytal intraperitoneally	−1 3	−1 0	−4 8	−21 0	−14 3	−5 5	−5 6	−3 2			
B. Samples taken one hour apart, 2 hours after urethane intraperitoneally	±0 8	±0 7	±2 0	−5 0		−2 0	−1 5				

sure of the dog to continued anesthesia, surgery, manipulation, and sampling.

The mean deviations representing each of the three types of errors are shown for eleven analyses in table 1. It is evident at once that the analyses of plasma and of whole blood were extremely accurate and reproducible, while the analyses of red corpuscles and of all other tissues were markedly less accurate. Of the solid tissues the liver gave by far the most reproducible samples. The difficulty of securing representative tissue samples is seen to be almost wholly physiological; this means simply that blood is the only tissue that continually mixes itself in the body, all other tissues are uniform neither in neighboring nor in symmetrical portions. It should be borne in mind that the deviations shown are for the ratio of liquid to solid constituents, these deviations being percentually 1.2 to 4

times as large as the deviations for the ratio of liquid to total. Duplicate analyses of all tissues except skin were on the latter basis accurate to ± 0.6 per cent.

Possible effects of heparin upon the analyses were studied in detail, since it was impracticable to dissolve the same concentration of heparin in each blood sample. It is well known (Fischer, 1932) that the refractive index of plasma is changed by adding heparin, this change affecting likewise any fractionation of globulin and albumin. Mr. A. A. Parry kindly compared the effect of adding crude heparin (Hynson, Westcott & Dunning) to fresh dog's blood and serum in concentrations up to the maximum amount used in preserving samples in our experiments. An increase of refractive index could regularly be demonstrated; the maximal range in the concentration of heparin used (between 0.01 per cent and 0.05 per cent in whole blood) produced an effect equivalent to an apparent decrease of fluid ratio of the plasma (*PR*) amounting to 1.6 per cent. This is twice the mean error actually found among successively drawn heparinized samples (table 1) and we believe this error was never present. In any case, no conclusions were drawn from single samples showing changes of less than this magnitude.

The errors in specific gravity and in dry residue in plasma due to adding heparin were of course smaller than the errors in duplicate analyses. The analyses of dry residue in blood plasma (*PW*) were highly variable because of the small amounts used in the analyses. In addition, evaporation slowly occurred during centrifugation, in spite of the fact that the centrifuge tubes were capped with rubber nipples throughout their manipulation. This one (*PW*) was the only type of analysis not regularly done in duplicate or triplicate.

It was found that the changes of hydration after hemorrhage in all tissues except blood are less than the errors of analysis. The changes in muscle are too small to measure in all procedures that have been used except intravenous infusions. Various investigators have stated average amounts of change in water content of tissues sampled *in vivo* following hemorrhage (Underhill and Fisk, 1930; Harris and Blalock, 1931; Robinson and Parsons, 1931). We are unable to report significant changes of water content in muscle or in skin.

Our control data show that the factor of time elapsed during the experiment is most important in changing tissue hydrations, as Harris and Blalock (1931) also noticed. It is apparently impossible to keep an operated animal free of evaporation, and likewise impossible to keep the rates of evaporation equal in various animals. It is not feasible to restore the fluid supply at a constant rate. Hence in one hour all tissues in the body including whole blood have lost amounts of water equal to 1 to 5 per cent of their weights. The plasma often meanwhile even gained water in slight

amounts because of the hemorrhage involved. The loss of water from the whole blood was significantly greater when solid tissues were being exposed and sampled. Due to this factor of time and manipulation it is rarely practicable to follow changes of hydration due to any procedures but acute ones.

HEMORRHAGE. The amount of hemorrhage induced varied from 20 to 35 cc. per kilogram of body weight in different dogs. This volume of blood was drawn from the cut artery in a period varying from 4 to 20 minutes. The rapidity of blood dilution appeared to be uncorrelated with the duration of the bleeding within these limits.

All the hemorrhage experiments are listed in table 2. They were of two sorts: on whole dogs, of which two were splenectomized, and on "fore-third" preparations.

TABLE 2

Summary of hemorrhage experiments

1	2	3	4	5	6	7	8
DOG NUMBER	BODY WEIGHT	HEMORRHAGE	ANESTHETIC	OPERATION	TISSUES SAMPLED	DURATION OF HEMORRHAGE	RECOVERY FOLLOWED
	kgm.	*cc./kgm.*				*minutes*	*minutes*
De.....	17.8	23	Amytal	Usual	*B, M*	8	70
Dg.....	17.1	20	Amytal	Usual	*B, M, S*	10	23
Dv.....	22.0	35	Amytal	Usual	*B, M, S, L*	4	30
Fa.....	24.8	30	Amytal	Usual	*B*	20	129
Ff.....	26.3	23	Amytal	Splenec.	*B*	15	60
Fi.....	18.4	32	Urethane	Usual	*B, L*	17	21
Fh.....	10.6	23	Urethane	Splenec.	*B*	20	62
Fk.....	18.5	9 (or 27)	Urethane	Fore-third	*B*	4	9
Fm.....	16.9	2 (or 6)	Urethane	Fore-third	*B*	2	23

Whole animal. Seven experiments belong to this series, five under amytal anesthesia and two under urethane. The responses obtained may be followed in figure 1, which represents one of the amytal experiments.

The circulatory responses consisted primarily in falls of all pressures—systolic arterial, diastolic arterial, mean arterial, mean venous, and by inference mean capillary. The arterial pulse pressure usually fell too. At the close of the bleeding period the arterial pressures tended to recover, but only slightly and slowly. The pulse rate increased; this may be regarded as a response compensatory to the pressure changes.

The changes in blood concentration are primarily of two kinds, those characterizing the corpuscles and those characterizing the plasma. The corpuscles (*BT, BH*) exhibit a marked and sudden concentration, followed by a slow dilution which may begin before bleeding has ceased. This

change in concentration of corpuscles in the circulating blood occurred in three of the four experiments upon whole animals with intact spleens under amytal anesthesia. It did not occur under this anesthesia when the spleen had first been removed (expt. Ff). This response of the corpuscular elements is due to discharge of the spleen, this discharge occurring with great regularity in response to hemorrhage (Barcroft *et al.*, 1925). Previous investigators have noted (Binet and Fournier, 1926), and our

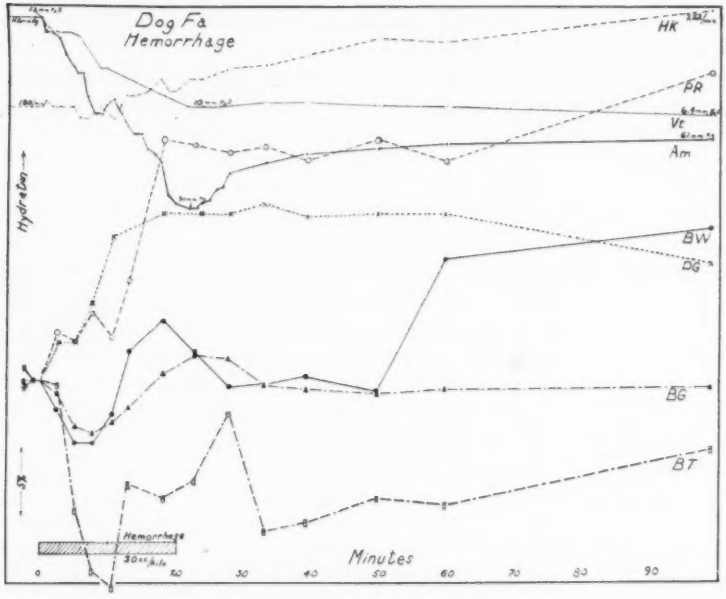

Fig. 1. Changes of blood pressures and of blood dilutions before, during, and after hemorrhage of 750 cc. in dog Fa, under amytal anesthesia. *Hk*, heart rate in beats per minute, *Vt*, venous pressure in the right femoral vein, *Am*, mean mercury pressure in the left carotid artery; *PR*, dilution ratio of plasma refractive index, *BW*, of whole blood water content, *PG*, of plasma specific gravity, *BG*, of whole blood specific gravity, *BT*, of hematocrit. For each ratio the second blood sample is taken as 100 per cent.

experiments show, that the corpuscles added to the circulating blood may be more than enough to counterbalance the entire increase in plasma volume that later progressively occurs. One of the effects of amytal anesthesia is to remove corpuscles from circulation and thereby enlarge enormously the splenic store (Adolph and Gerbasi, 1933). Hence the response in the corpuscular elements to hemorrhage is probably much greater in the amytalized than in the unanesthetized animal. That the corpuscles

stored in the spleen are sufficient to change the concentration of the blood by the amounts found, are shown by the calculations of table 3. In these calculations it is assumed that 8 cc. per kilogram of body weight (Adolph and Gerbasi, 1933) were discharged by the spleen into 90 cc. of circulating blood that contained 54 cc. of plasma; the splenic blood and the circulating blood were actually analysed.

Under urethane anesthesia, the whole animal, even with spleen intact (expt. Fi), exhibited no increased concentration of corpuscles during hemorrhage. This is correlated with the fact that when urethane is administered intraperitoneally the spleen remains discharged and the circulating blood is rich in corpuscles (Adolph and Gerbasi, 1933). Evidently the spleen does not discharge further; in fact there is not enough blood left in it to be discharged if it were stimulated to do so. Further, the response to hemorrhage under urethane was essentially unchanged after splenectomy (expt. Fh). The urethanized dogs died from respiratory failure about an hour after 25 cc. of blood per kilogram of body had been removed, while amytalized dogs withstood even more bleeding without critical detriment.

TABLE 3

Percentage changes in dilution found in circulating blood compared with those expected from discharge of the amytalized spleen

DOG NUMBER	TIME OF SAMPLING BLOOD	*PR*	*BT*	*BH*	*BG*
	minutes	*per cent dilution*	*per cent dilution*	*per cent dilution*	*per cent dilution*
Observed:					
Dg	15	+2.6	−9.8	−11.9	−0.3
Dv	2	+3.2	−13.0	−11.2	−2.8
Fa	7	+4.9	−14.0	−2.8	−4.0
Calculated:					
Ff	–	−0.2	−12.9	−4.4	−2.3
Fg	–	−0.5	−12.0	−5.4	−2.4

In hemorrhage the amytalized animal with spleen distended has a reserve of blood available. The urethanized animal with spleen emptied not only has no splenic reserve, but loses 40 to 50 per cent more corpuscles in every drop of blood lost. The whole body acts as a reservoir for plasma, but for corpuscles the spleen is the chief store.

The plasma exhibited marked dilution in all hemorrhage experiments. This dilution began with the first minute of hemorrhage, and progressed throughout its duration and for a time after bleeding has ceased. This is shown for all experiments in figure 2. In several experiments this dilution followed an exceedingly uniform curve which was very nearly a straight

line during the first ten or twenty minutes. In some experiments the initial dilution ceased abruptly when the bleeding ceased, but when the bleeding was very rapid the initial dilution much outlasted it, proceeding at a rate that progressively diminished.

The initial dilution was completed in 15 to 20 minutes in a majority of the experiments (fig. 2). Following it there was usually a slight concentration of the plasma, which may correspond to the immediate increase of mean capillary pressure at the cessation of bleeding. After this brief phase of concentration, the plasma was again diluted continuously and slowly. In one experiment, Fh, the continued dilution was quite rapid, lasting for at least one hour.

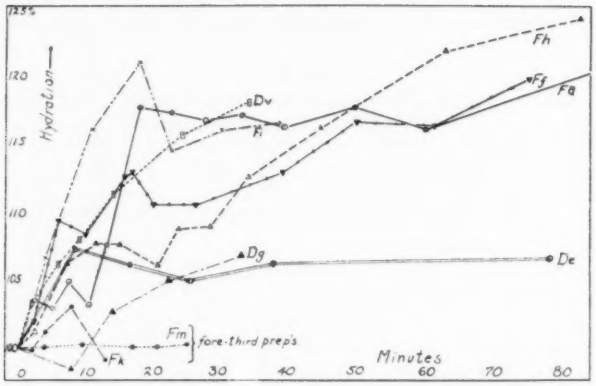

Fig. 2. Changes of dilution ratios as calculated from determinations of plasma refractive index during and after hemorrhage, in each of the nine experiments. The amounts and durations of hemorrhage are listed in table 2. In each experiment hemorrhage began at zero time, and the dilution ratio (*PR*) found in the blood sample taken just before this time was taken as 100 per cent.

The increase of plasma dilution may be regarded as a measure of the increase of plasma volume. This relation rests on the assumption that protein is neither added to nor subtracted from the circulating plasma in significant amounts; no evidence is available against this assumption, which will be discussed below. This relation allows comparison of the amounts of fluid restored to the plasma with the amounts of plasma removed by bleeding. Table 4 shows the calculation for each of the hemorrhage experiments. From figure 2 are obtained the data for columns 3, 7, and 9. Column 5 is readily calculated from column 4 taken together with column 3 of table 2. Approximate values for the volume of plasma originally in the animal are obtained by allowing 50 cc. per kilogram of body weight in the normal dog (Smith *et al.*, 1921), 54 cc. in the amytalized

dog, and in the urethanized dog 49 cc. if the anesthetic is administered by stomach tube (as in Fh) and 46 cc. if administered intraperitoneally. The small differences used in these estimates are based on considerations found in the paper of Adolph and Gerbasi (1933); from them are derived the data for column 6. Table 4 shows that the primary mean dilution is sufficient to restore *35 per cent of the plasma removed.*

Undoubtedly the blood volume is considerably filled out by the corpuscles that are added from the spleen in those experiments in which the spleen was able to deflate. Possibly for this reason the plasma dilution was less in the experiments done under amytal than in those done under urethane.

TABLE 4

Restoration of fluid in the blood plasma

1	2	3	4	5	6	7	8	9
DOG NUMBER	DURATION OF HEMORRHAGE	DURATION OF INITIAL RESTORING DILUTION OF PLASMA	HEMATOCRIT OF BLOOD REMOVED	VOLUME OF PLASMA REMOVED	RATIO OF PLASMA VOLUME REMOVED TO PLASMA VOLUME REMAINING	INCREASE OF PLASMA DILUTION AT TIME 3 OVER INITIAL PLASMA DILUTION	RATIO OF PER CENT DILUTION TO PER CENT PLASMA REMOVED	RATE OF PLASMA DILUTION IN FIRST TEN MINUTES
	minutes	*minutes*	*per cent R.B.C.*	*cc./kgm.*	*per cent*	*per cent*	*per cent*	*per cent per minute*
De	8	8	35	15	38	7	18	0.8
Dg	10	33	49	10	23	7	30	0.5
Dv	4	36	40	21	64	18	28	0.9
Fa	20	18	39	18	50	17	34	0.6
Ff	15	15	48	12	29	12	41	1.0
Fi	17	18	67	11	31	20	65	1.5
Fh	20	23	52	11	29	9	31	0.7
Fk	4	8	69	9	24	3	12	0.3
Fm	2	0	55	2	5	0	0	0
Mean of first seven	13	22		14	38	13	35	0.8

It might be supposed that the corpuscles in circulation would swell, their constituents suffering dilutions equivalent to those of the plasma. To find if this were true, the changes in ratio of hematocrit dilution to hemoglobin dilution were calculated in each experiment. It was found that the relative corpuscular volumes diminished during hemorrhage in as many experiments as they increased. But in each experiment the diminution or the increase prevailed throughout the period of hemorrhage, usually approaching normal early in the recovery period. Of course the carbon dioxide tension was not controlled in the samples of blood that were being analysed; yet all were treated alike as far as could be done; all were taken serially in performing each analysis. In hemorrhage new small cells of

diverse hemoglobin concentrations are sometimes supplied to the circulation from the bone marrow; the effect of these on the corpuscular dilutions can only be surmised.

The dilution of the plasma in hemorrhage can be readily accounted for by observing its correlation with the changes of blood pressure. Hemorrhage is characterized by a lowering of all vascular pressures. This diminishes the tendency of fluid to be squeezed out of the blood plasma as it passes through the capillaries. Hence what is essentially a salt solution enters the blood, its entrance being now less opposed by the hydrostatic pressure within the blood. Dilution ceases when a new equilibrium of hydrostatic and osmotic forces is reached. This is the picture presented by Starling's (1896) conception of fluid interchanges in the body.

The rate of entrance of fluid into the blood is readily obtained from the plasma dilution curves of figure 2. The values found are shown in the last column of table 4. The mean rate of 0.8 per cent of the plasma volume per minute represents an entrance of 0.25 cc. per kilo of body weight per minute. This corresponds to a rate of transudation that Krogh, Landis, and Turner (1931) believed they could demonstrate in the human arm tissues under a venous stasis of 28 cm. of water. It is conceivable that in our dogs the mean capillary pressure was decreased by some such amount during hemorrhage; the mean venous pressure actually was decreased by 2 to 5 centimeters of water.

Fore-third preparation. A major objective in this study was to find whether the dilution of the blood in hemorrhage was local or general in occurrence. Effort was directed toward discovering whether the viscera played a special rôle. It was believed that the best method of eliminating the viscera from consideration in a hemorrhage experiment was to clamp the aorta and the vena cava above the diaphragm. This was done in two hemorrhage experiments, both under urethane anesthesia (Fk, Fm). It is believed that close to one-third of the tissue mass is left in contact with the circulating blood by this operation.

Such preparations continued in good circulatory and respiratory condition for an hour or more (expts. Dz, Eb, Fd). Artificial breathing was given while hemostats were introduced through the chest wall; after the clamping the chest was sealed off by means of the skin and natural breathing was resumed. Due to the diminished periphery now presented to the unchanged heart and lungs, the circulation was extremely modified. The arterial pulse pressure suffered a sudden decrease, at first by a decrease in systolic pressure with an increase in diastolic pressure. These pressure responses could be varied by clamping the vena cava at various intervals after clamping the aorta, since the fore third of the body was furnished varying amounts of blood by this means. After the initial adjustment, the mean arterial pressure fell quite rapidly, though a new level might be maintained for some time, below the level for the whole animal.

The amount of hemorrhage endured by such a preparation was extremely small. After only 6 cc. per kilogram of the weight of the fore third had been bled, breathing promptly ceased. Artificial breathing by rhythmic insufflation enabled the animal to survive only about ten minutes, when the heart failed.

Dilution of the blood following hemorrhage occurred in one fore-third preparation (Fk). It was slow and small compared to the dilution in the whole animal. The ratio of blood to other tissues was little modified by the double occlusion; hence the slow dilution may possibly indicate that the viscera play a quantitatively special rôle in transudation. The experiments were made less conclusive by their early termination, and by the fact that the vascular pressures fell progressively as a result of the operation alone. In neither fore-third experiment was the concentration of corpuscles increased by hemorrhage.

COMMENT. In the course of this investigation it became evident that true fluid exchanges of blood can be measured in most cases only in relation to the concentration of the plasma. So long as the spleen is in the body, corpuscular constituents and whole blood form uncertain bases of reference for water contents; and it is probable that other tissues such as the liver (Grab *et al.*, 1929; Bauer *et al.*, 1932) and the skin (Barcroft *et al.*, 1932) may in smaller degree behave like the spleen. Since most inorganic materials pass in and out of the blood plasma with the water, it is necessary to refer changes of concentration to the proteins of the plasma. Precisely this basis chiefly prevails in the determinations of refractive index, dry residue, and specific gravity of plasma.

Attempts to locate changes of water content in the solid tissues failed. Our analyses of muscle and of skin were clearly insignificant, and previous investigators (Underhill and Fisk, 1930; Harris and Blalock, 1931; Robinson and Parsons, 1931) who analysed muscle after hemorrhage hardly proved that the losses of water observed by them were significant. The last named used conditions of experiment more comparable to ours, and found signs of reversible dehydration of muscle. In one (Dv) of two experiments in which samples of liver were taken, we found a substantial progressive dehydration of liver substance, amounting to a decrease in hydration (*LW*) of 6 per cent during the hemorrhage and 12 per cent after it. Whether this loss of fluid represented intercellular or intracellular media is uncertain; it could not be ascribed to changes of whole blood content of the liver samples. Evidently the change in the liver was less reproducible than the change of hydration in the blood plasma.

It must be recalled in interpreting the experiments that progressive changes occur in the blood with no procedures other than those of operation, exposure, and sampling. One thing that accompanies these procedures is hemorrhage itself. All the operations were ordinarily performed

with a loss of circulating blood amounting to less than 10 cc., even including incision of the abdomen. Every blood sampling required 5 to 7 cc. of blood, which is 0.3 to 0.4 per cent of the blood volume. Every sampling of liver lost with it at least this much blood, of the muscle somewhat less blood, and of the skin practically no blood. For this reason, and because excessive exposure to evaporation was avoided, the experiments in which only blood was sampled are the more reproducible. In the experimental data as presented, no allowance was made for the additional hemorrhage due to sampling; in control experiments the effects of this loss of blood were just about sufficient to compensate for the increase of *plasma* concentration that would occur due to duration of exposure.

The conception of Starling (1896) has been found widely useful in combining the factors underlying exchanges of water between blood and extravascular spaces. The factor that has taken precedence in recent work is that of the colloid osmotic pressure of the blood. But the factor of the mean pressure of the blood in the capillaries is of equal importance. We have tested a number of procedures whereby the pressure of the blood was varied, in the same manner as the effect of hemorrhage was tested (Adolph and Lepore, 1931). These experiments show that bleeding is one of the most effective ways of lowering the capillary pressure; and that it is at the same time one of the most effective ways of moving water from outside the blood vessels into the circulating blood. Obviously the change of hydrostatic pressure and the movement of water are at least in part cause and effect.

At present there is no means of measuring the particular mean capillary pressure that controls water movement except by the movement itself. It is highly uncertain what rates of water exchange occur in the various portions of a single capillary (Landis, 1930; Rous *et al.*, 1930). Hence cause and effect cannot as yet be further quantitatively correlated.

There is evidence of long standing that changes of mean arterial blood pressure go hand in hand with changes of blood concentration. Cohnstein and Zuntz (1888), Sherrington and Copeman (1893), and Starling (1894) adduced evidence that such is the case, which was later amplified by Mummery and Symes (1908), Scott (1917), and others. Apparently, however, the changes of pressure that are effective in the *capillaries* which can be produced by most procedures are relatively small compared to those which can be produced by hemorrhage.

In addition to the large decrease of mean capillary blood pressure, there is in hemorrhage a sharp decrease in the oxygen being carried to the capillaries. Whether there is a lack of oxygen sufficient to influence water exchanges is doubtful. If there is, the factors of acidity and of increased permeability of capillaries to plasma proteins become outstanding. No reliable evidence for modifications of water exchange with acid accumula-

tion in tissues has been uncovered under strictly physiological conditions, but this by no means precludes their existence. That plasma proteins leave the blood plasma more extensively when there is lack of oxygen has been noted in a number of studies, especially under the influence of stasis or of histamine; but it is notable that in both these circumstances the local capillary blood pressure is increased too. The loss of protein from the circulating plasma quickly reduces the effective colloid osmotic pressure of the blood. In addition Landis (1928) measured directly on the frog the increased rate of water transport out of mesenteric capillaries in the absence of oxygen. It is doubtful whether any measurable lack of oxygen exists in ordinary hemorrhage (Gollwitzer-Meier, 1928; Blalock, 1927).

We believe there is little *loss* of protein from the circulating plasma in simple hemorrhage because 1, the ratio of plasma proteins to hemoglobin in samples of circulating blood as drawn from splenectomized dogs was not diminished, and 2, the albumin-globulin ratio in the circulation increases within 5 hours rather than decreases (Inagaki, 1907). The latter change may possibly betoken a *gain* of tissue-fluid protein by the circulation, increasing the plasma's total attraction for water, whether it pours through the walls of the tissue capillaries or through the lymphatic system; but this is utterly unlikely within the initial period of our experiments.

This gain would have made the percentage volume of fluid entering the circulation somewhat higher than was indicated by the percentage dilution of the plasma that was measured.

The blood volume determinations that were done within a few minutes after hemorrhage was produced in dogs by Carrier, Lee and Whipple (1922) did not show significantly increased plasma volumes. The increase expected in 22 minutes on the basis of table 4 would be about 9 per cent of the *initial* plasma volume.

Urinary losses of water could have had little influence during the brief period of time over which the changes of blood concentration due to hemorrhage were followed. They were of course included in the changes found in the control experiments in which no systematic hemorrhage occurred.

The changes of blood concentration that occur in hemorrhage have been frequently studied. In most instances some constituent of corpuscles or of whole blood was used as criterion of this change, a criterion that, as we have seen, immensely complicates and varies the results found. Dilution of plasma or of serum has been previously demonstrated within a few minutes or hours after hemorrhage by means of refractive index (Schöneich, 1906; Inagaki, 1907; Oliva, 1911; Reiss, 1913; Behrens, 1928), specific gravity (Zimmermann, 1845; Inagaki; Oliva; Kerr, 1926; Moore and Stewart, 1930), and dry residue (Zimmermann; Kerr). But in no instances were analyses repeated at brief intervals to determine how soon and how rapidly these changes occurred.

The immediate physiological effects of hemorrhage may be summarized from the literature in the following way: the primary effects of loss of blood from circulation are to decrease the arterial, venous, and capillary pressures, and to decrease the rate of blood flow and oxygen flow to most tissues. To this situation the circulation responds by increase in heart rate, discharge of adrenalin, discharge of blood from the spleen, local vasoconstrictions, and local persisting ischemias. The respiration responds to the local asphyxia in its centers and to the pouring of lactate into the circulation by hyperventilation, followed eventually by asphyxia and cessation of breathing. In the capillaries fluid flows into the blood from tissue spaces, water excretion by the kidneys is diminished, and various dissolved constituents interexchange in response to the lowered pressures, to the anoxia, and to the altered types of tissue metabolism.

SUMMARY

Changes in concentration in blood, and sometimes in other tissues, were measured at intervals of a few minutes before, during, and after severe hemorrhage in anesthetized dogs.

Initial increases of concentration of corpuscles were prevented by previous removal or deflation of the spleen.

Dilution of the plasma was always observed. It is due to the migration of fluid from extravascular spaces into the circulating plasma. The mean rate of this dilution amounted initially to about 0.25 cc. per kilo per minute, and required about 22 minutes for approximate completion.

In the absence of the viscera the rate of dilution was greatly reduced.

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